

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

Bayer 10,203-KGB

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/424686

INTERNATIONAL APPLICATION NO.

PCT/EP98/03468

INTERNATIONAL FILING DATE

09 June 1998 (09.06.98)

PRIORITY DATE CLAIMED

20 June 1997(20.06.97) 14 April 1998(14.04.98)
26 March 1998(26.03.98)

TITLE OF INVENTION HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS DIAGNOSTIC AND
THERAPEUTIC USE

APPLICANT(S) FOR DO/EO/US

SEE ATTACHED APPENDIX

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - a) Copy of the WO-Publication (WO 98/59040)
 - b) Copy of the PCT-Request (PCT/RO/101)
 - c) Copy of the search report (PCT/ISA/210) in English and German
 - d) Copy of the examination report (PCT/IPEA/409) in German
 - e) Appendix with Express Mail Label No.
 - f) Figs. 1-19
 - g) Notice of Change of Firm Name

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PCT/EP98/03468

17. ☒ The following fees are submitted:**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO \$970.00

International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO \$840.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but
international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$760.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 840.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	13 - 20 =		X \$18.00
Independent claims	1 - 3 =		X \$78.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00

\$

\$

\$

TOTAL OF ABOVE CALCULATIONS =

\$ 840.00

Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement
must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

\$

SUBTOTAL =

\$ 840.00

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

+

TOTAL NATIONAL FEE =

\$ 840.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

\$

+

TOTAL FEES ENCLOSED =

\$ 840.00

Amount to be:

refunded

\$

charged

\$ 840.00

a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 14-1263 in the amount of \$ 840.00 to cover the above fees.
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 14-1263. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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33,141

REGISTRATION NUMBER

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420 Rec'd PCT/PTO 29 NOV 1999

Attorney Docket No. :Bayer 10,203-KGB:pb
:Le A 32 486-US KK/Sto/gp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : GUSTAV HAGEN ET AL.
Serial No. : TO BE ASSIGNED
Filed : HEREWITH
For : HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS
DIAGNOSTIC AND THERAPEUTIC USE
Art Unit : TO BE ASSIGNED
Examiner : TO BE ASSIGNED

November 29, 1999

Hon. Assistant Commissioner
for Patents
Washington, D. C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 3, delete "compounds" and substitute -- telomerase --; and delete "Claims" and substitute -- Claim --; and delete "and 2".

Claim 5, after "3" insert a period and cancel the balance of the claim.

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Claim 6, delete "Claims 1 and 2," and substitute -- Claim 1--.

Claims 7-9, delete "Use of" and substitute -- Method of using --.

Claim 7, delete "Claims 3 and 4" and substitute -- Claim 3 --.

Claim 10, delete "Claims 3 and 4" and substitute --Claim 3 --.

Claim 12, delete "Claims 1 and 2" and substitute -- Claim 1 --.

Claim 13, delete "the telomerase according to Claims 1 and 2" and substitute -- a telomerase --.

REMARKS

The foregoing amendments remove multiple dependencies and otherwise place the claims in better form for U.S. examination.

GUSTAV HAGEN ET AL.

Early and favorable action is earnestly solicited.

Respectfully submitted,
NORRIS/McLAUGHLIN & MARCUS

By


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- 1 - 420 Rec'd PCT/PTO 29 NOV 1999

Catalytic subunit of human telomerase and its diagnostic and therapeutic use**Structure and function of the chromosome ends**

5 The genetic material of eukaryotic cells is distributed on Linear chromosomes. The ends of these hereditary units are termed telomeres, derived from the Greek words *telos* (end) and *meros* (part or segment). Most telomeres consist of repeats of short sequences which are mainly constructed from thymine and guanine (Zakian, 1995). The telomere sequences of related organisms are often similar and these sequences are even conserved between species
10 which are more phylogenetically remote. It is a remarkable fact that the telomeres are constructed from the sequence TTAGGG in all the vertebrates which have so far been examined (Meyne *et al.*, 1989).

15 The telomeres exert a variety of important functions. They prevent the fusion of chromosomes (McClintock, 1941) and consequently the formation of dicentric hereditary units. Chromosomes of this nature, possessing two centromeres, can lead to the development of cancer due to loss of heterozygosity or the duplication or loss of genes.

20 In addition, telomeres serve the purpose of distinguishing intact hereditary units from damaged hereditary units. Thus, yeast cells ceased dividing when they harboured a chromosome which lacked a telomere (Sandell and Zakian, 1993).

25 Telomeres carry out another important task in association with DNA replication in eukaryotic cells. In contrast to the circular genomes of prokaryotes, the Linear chromosomes of eukaryotes cannot be completely replicated by the DNA polymerase complex. RNA primers are required for initiating DNA replication. After the RNA primers have been eliminated and the Okazaki fragments have been extended and then ligated, the newly synthesized DNA strand lacks the 5' end because the RNA primer at that point cannot be replaced with DNA.
30 For this reason, without special protective mechanisms, the chromosomes would shrink with every cell division ("end-replication problem", Harley *et al.*, 1990). The non-coding telomere

sequences probably represent a buffer zone for preventing the loss of genes (Sandell and Zakian, 1993).

Over and above this, telomeres also play an important role in regulating cell ageing (Olovnikov, 1973). Human somatic cells exhibit a limited capacity to replicate in culture; after a certain time they become senescent. In this condition, the cells no longer divide even after being stimulated with growth factors; however, they do not die but remain metabolically active (Goldstein, 1990). Various observations provide support for the hypothesis that a cell determines from the length of its telomeres how often it can still divide (Allsopp *et al.*, 1992).

In summary, the telomeres consequently possess central functions in the ageing of cells and in the stabilization of the genetic material and prevention of cancer.

The enzyme telomerase synthesizes the telomeres

As described above, organisms possessing Linear chromosomes are only able to replicate their genomes incompletely in the absence of a special protective mechanism. Most eukaryotes use a special enzyme, i.e. telomerase, to regenerate the telomere sequences. Telomerase is expressed constitutively in the single-cell organisms which have so far been examined. By contrast, in humans, telomerase activity was only detected in germ cells and tumour cells whereas neighbouring somatic tissue did not contain any telomerase (Kim *et al.*, 1994).

Telomerase in ciliates

Like the telomeres, telomerase was identified for the first time in the ciliate *Tetrahymena thermophila*. Telomerase activity was detected by extending the single-stranded oligonucleotide d(TTGGGG)₄ in the presence of dTTP and dGTP (Greider and Blackburn, 1985). In this reaction, the *Tetrahymena* telomere sequence TTGGGG was added repeatedly to the primer. Even when an oligonucleotide having the irregular telomere sequence of *Saccharomyces cerevisiae*, T(G)₁₋₃, was offered as the starting material, the telomerase

extended the primer with the telomere sequence of *Tetrahymena* (Greider and Blackburn, 1985). From these results, it was concluded that the telomerase itself carries the template for the sequence of the telomeres.

5 Once the existence of an RNA component in the telomerase had initially been demonstrated (Greider and Blackburn, 1987), the gene for the RNA subunit of the telomerase was cloned a short while later (Greider and Blackburn, 1989). This RNA contains a region which is complementary to the *Tetrahymena* telomere sequence (termed "complementary region" below). The activity of the telomerase depended on the RNA component, as was
10 demonstrated by digesting the RNA, leading in turn to subsequent loss of activity. If the complementary region of the telomerase RNA was mutated, the corresponding mutations were incorporated *in vivo* into the *Tetrahymena* telomeres (Yu *et al.*, 1990). Telomerase consequently belongs to the class of RNA-dependent DNA polymerases.

15 The first protein subunits of the *Tetrahymena* telomerase, i.e. p80 and p95, were identified in 1995 (Collins *et al.*, 1995). The observation that p95 anchors the enzyme to the DNA and p80 binds the RNA component led to the following model: the telomerase RNA anneals by its complementary region to the single-stranded 3' overhang. The 3' overhang is extended by incorporating the corresponding nucleotides in the 5'-3' direction. The *de novo* synthesis of
20 telomeres probably involves an elongation step and a translocation step. Once a telomere sequence has been synthesized, the telomerase presumably moves along the DNA until it is once again in a position to be able to add a complete telomere sequence. This model does not have to be generally valid since great differences exist between the telomerases of different species with regard to the number of nucleotides which the enzyme adds before it dissociates
25 from the telomere (Prowse *et al.*, 1993).

In addition to this, telomerase subunits from other organisms have also recently been identified. Two protein subunits, i.e. p123 and p43, which do not exhibit any homology with the *Tetrahymena* telomerase proteins, have been found in the ciliate *Euplotes aediculatus*.
30 The telomerase subunit p123 exhibits a basic domain at its N terminus and a domain for a reverse transcriptase (RT) at the C terminus, suggesting this protein has a catalytic function,

(Lingner *et al.*, 1997). Furthermore, p123 has been reported to share significant homology with the *Saccharomyces cerevisiae* protein Est2 which was found by Lundblad (Lingner *et al.*, 1997).

- 5 Whereas p80 and p95 have not hitherto been demonstrated to possess any function which is essential for telomerase activity, the potential catalytic telomerase subunits p123/est2p have been unambiguously shown to have a key function: mutation of the active centre of the est2p RT led to significant truncation of the telomeres in yeast cells (Lingner *et al.*, 1997).

10 Telomerase components from mammalian cells

15 The RNA components of the telomerases of various organisms, inter alia of *Saccharomyces cerevisiae*, mice and humans (Singer and Gottschling, 1994; Blasco *et al.*, 1996; Feng *et al.*, 1995), have by now been cloned. All the telomerase RNAs known to date comprise a region which is complementary to the telomere sequence of a particular organism. However, the primary sequence of the human telomerase RNA (hTR) does not display any similarity to the RNA components of the ciliates or of *Saccharomyces cerevisiae*. On the other hand, regions exist which are conserved between human and murine telomerase RNA (Feng *et al.*, 1995).

20 The isolation of a human telomerase-associated protein (hTP1) has recently been described (Harrington *et al.*, 1997). On the basis of its homology with the *Tetrahymena* telomerase p80 subunit, the corresponding gene was found in an EST data base which is not available to the general public (Harrington *et al.*, 1997). hTP1 is composed of 2627 amino acids and, in the N-terminus, exhibits three domains which possess at most 46% homology with p80. 16
25 repeats of the amino acids tryptophan and asparagine, which presumably mediate a protein/protein interaction, were shown to be present, as an additional structural element, in the C-terminal region.

30 Activation of the telomerase in human tumours

In humans, it was originally only possible to demonstrate telomerase activity in germ line cells and not in normal somatic cells (Hastie *et al.*, 1990; Kim *et al.*, 1994). After a more sensitive detection method had been developed (Kim *et al.*, 1994) a low level of telomerase activity was also detected in hematopoietic cells (Broccoli *et al.*, 1995; Counter *et al.*, 1995; Hiyama *et al.*, 1995). However, these cells nevertheless exhibited a reduction in the telomeres (Vaziri *et al.*, 1994; Counter *et al.*, 1995). It has still not been clarified whether the quantity of enzyme in these cells is insufficient to compensate for the telomere loss or whether the measured telomerase activity stems from a subpopulation, e.g. of incompletely differentiated CD34⁺38⁺ precursor cells (Hiyama *et al.*, 1995). In order to clarify this point, it would be necessary to detect the telomerase activity which was present in a single cell.

Interestingly enough, however, significant telomerase activity has been detected in a large number of the tumour tissues which have been tested to date (1734/2031, 85%; Shay, 1997), whereas no activity has been found in normal somatic tissue (1/196, <1%, Shay, 1997). In addition, a variety of investigations demonstrated that the telomeres continued to shrink in senescent cells which were transformed with viral oncoproteins and that it was only possible to find telomerase in the subpopulation which survived the growth crisis (Counter *et al.*, 1992). The telomeres were also stable in these immortalized cells (Counter *et al.*, 1992). Similar findings derived from investigations in mice (Blasco *et al.*, 1996) support the assumption that reactivation of the telomerase is a late event in tumorigenesis.

Based on these results, a "telomerase hypothesis" was developed which links the loss of telomere sequences and cell ageing to telomerase activity and the genesis of cancer. In long-lived species such as humans, the shrinking of the telomeres can be regarded as a tumour suppression mechanism. Differentiated cells, which do not contain any telomerase, cease dividing when the telomeres have reached a particular length. If such a cell mutates, a tumour can only develop from it if the cell is able to extend its telomeres. Otherwise, the cell would continue to lose telomere sequences until its chromosomes became unstable and it finally died. Reactivation of the telomerase is presumably the main mechanism which tumour cells deploy in order to stabilize their telomeres.

It follows from these observations and ideas that it should be possible to develop a therapy for tumours based on inhibiting telomerase activity. Conventional cancer therapies using cytostatic agents or short-wave irradiation damage all the dividing cells in the body in addition to damaging the tumour cells. However, since it is only germ line cells which contain significant telomerase activity, apart from tumour cells, telomerase inhibitors would attack the tumour cells more specifically and consequently evoke fewer undesirable side effects. Since telomerase activity has been detected in all the tumour tissues tested to date, it would be possible to employ these therapeutic agents against all types of cancer. The effect of telomerase inhibitors would then set in when the telomers of the cells had shortened to such an extent that the genome had become unstable. Since tumour cells usually exhibit shorter telomeres than do normal somatic cells, it would be cancer cells which would first of all be eliminated by telomerase inhibitors. By contrast, cells possessing long telomeres, such as the germ cells, would not be damaged until a much later stage. Telomerase inhibitors consequently represent an approach which points the way forward for cancer therapy.

However, it will only be possible to provide unambiguous answers to questions regarding the nature and the points of attack of physiological telomerase inhibitors when the protein structures of the enzyme, together with their functions, have also been identified and a deeper understanding of the various telomere-binding proteins has been obtained.

The invention relates to the catalytically active human telomerase subunit (phTC), where appropriate in purified form, to active moieties of the protein, to modulators, in particular agonists of the protein, to substances which imitate the function of the protein and to combinations of these components.

The invention furthermore relates to:

- The nucleic acid sequence which encodes the human protein phTC, specifically:
 - the genomic sequence of the hTC gene,
 - the cDNA sequence of the hTC gene,

- the DNA sequence of hTC variants
- the sequence of the mRNA which is transcribed from the hTC gene,
- parts of the abovementioned sequences, including the DNA sequence (SEQ ID No. 1) of hTC which is shown in Fig. 1.

5

- The nucleic acid sequences which encode hTC-homologous proteins in other mammals, specifically:

10

- the genomic sequences of hTC-homologous genes,
- the cDNA sequences of hTC-homologous genes,
- the sequences of the mRNAs which are transcribed from hTC-homologous genes,
- parts of the abovementioned sequences.

15

- Nucleic acid sequences which, in humans and other mammals, encode proteins which are related to the phTC protein, specifically:

20

- the genomic sequences of hTC-related genes in humans and other mammals,
- the cDNA sequences of hTC-related genes in humans and other mammals,
- the sequences of the mRNAs which are transcribed from hTC-related genes in humans and other mammals,
- parts of the abovementioned sequences.

25

- The above-described phTC protein, which is isolated from mammalian cells (cf. Fig. 2 and SEQ ID No. 2).

30

- The phTC protein which is labelled with a detection reagent, with the detection reagent preferably being an enzyme, a radioactively labelled element or a fluorescent chemical.
- An antibody which is directed against the phTC protein.

According to a preferred embodiment, this antibody is a polyclonal antibody.

According to another preferred embodiment, this antibody is a monoclonal antibody.

Antibodies of this nature can be produced, for example, by injecting a host, which is substantially immunocompetent, with a quantity of a phTC polypeptide, or a fragment thereof, which is effective for producing the antibody, and by subsequently isolating this antibody.

In addition, an immortalized cell line which produces monoclonal antibodies can be obtained in a manner known per se.

Where appropriate, the antibodies can be labelled with a detection reagent.

Fragments which possess the desired specific binding properties can also be employed instead of the complete antibody.

Preferred examples of such a detection reagent are enzymes, radioactively labelled elements, fluorescent chemicals or biotin.

Oligonucleotides in purified form which have a sequence which is identical or exactly complementary to a contiguous sequence, of from 10 to 500 nucleotides in length, of the above-described genomic DNA, cDNA or mRNA.

An oligonucleotide of this nature can, in particular, be an oligodeoxy-ribonucleotide or an oligoribonucleotide or a peptide nucleic acid (PNA).

Preference is given to oligonucleotides which inhibit, repress or block the activity of the telomerase when they bind to the hTC mRNA.

- A DNA sequence, or a degenerate variation of this sequence, which encodes the phTC protein, or a fragment of this protein, where appropriate comprising the DNA sequence in Figure 1a, or a DNA sequence which hybridizes with the previously cited DNA sequence under standard hybridization conditions.

5

- A recombinant DNA molecule which comprises a DNA sequence, or a degenerate variation of this sequence, which encodes phTC or a fragment of phTC, with the latter sequence preferably comprising the DNA sequence in Figure 1a, or which comprises a DNA sequence which hybridizes with the previously cited DNA sequence under standard hybridization conditions.

10

In the abovementioned recombinant DNA molecule, the described DNA is preferably linked to an expression control sequence.

15

Examples of expression control sequences which are particularly preferred are the early or late promoter of the SV40 virus or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the main operator and promoter regions of phage λ , the control regions of the fd coat protein, the 3-phosphoglycerate kinase promoter, the acid phosphatase promoter and the yeast α -mating factor promoter.

20

- A single-cell host which has been transformed with the above-described recombinant DNA molecule which comprises the DNA sequence, or a degenerate variation of this sequence, which encodes the phTC protein or a part of this protein. In this recombinant DNA molecule, the said DNA sequence is linked to an expression control sequence.

25

Preferred examples of the single-cell host are: *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40 and BMT10 cells, plant cells, insect cells and mammalian cells in cell culture.

30

- A recombinant virus which is transformed with one of the previously described DNA molecules or a derivative or fragment of this molecule.
- A method for inhibiting telomerase activity in human cells, preferably neoplastic cells, in which an exogenous polynucleotide which consists of a transcription unit is transferred into the cells. This transcription unit comprises a polynucleotide sequence of at least 29 consecutive nucleotides, which sequence is substantially identical or substantially complementary to the hTC RNA sequence and is linked to a heterologous transcription-regulating sequence which controls the transcription of the linked polynucleotide in the said cells.

Preferably, the abovementioned heterologous transcription-regulating sequence comprises a promoter which is constitutively active in human cells.

Alternatively, the heterologous transcription-regulating sequence can comprise a promoter which can be induced or repressed in human cells by adding a regulatory substance. Examples of such promoters are inducible and repressible tetracycline-dependent promoters, heat shock promoters and metal ion-dependent promoters.

The abovementioned exogenous polynucleotide can, for example, be a viral genome containing a transcription unit from the human hTC DNA component.

Particularly preferably, the said transcription unit produces antisense RNA which is substantially complementary to the human hTC RNA component.

Particular preference is also given to the exogenous polynucleotide being able to comprise the sequence in Fig. 1a.

- A polynucleotide for the genetic therapy of a human disease. This polynucleotide consists of a transcription unit which comprises a polynucleotide sequence of at least 9 consecutive nucleotides, which sequence is substantially identical or substantially

complementary to the hTC RNA sequence and is linked to a heterologous transcription-regulating sequence which controls the transcription of the linked polynucleotide in said cells.

5 - A method for detecting telomerase-associated conditions in a patient, which method comprises the following steps:

- 10 A. Detecting the phTC protein in body fluids or cell samples in order to obtain a diagnostic value;
- B. Comparing the diagnostic value with standard values for the phTC protein in standardized normal cells or body fluids of the same type as the test sample;
- C. Detecting diagnostic values which are higher or lower than the standard comparative values and which indicate a telomerase-associated condition, which condition in turn indicates a pathogenic condition.

15 This method is preferably employed for detecting a neoplastic disease in a patient. The method then comprises the following steps:

- 20 A. Detecting the phTC protein in cell samples in order to obtain a diagnostic value;
- B. Comparing the diagnostic value with standard values for the phTC protein in non-neoplastic cells of the same type as the test sample;
- C. Diagnostic values which are clearly higher than standard comparative values indicate a neoplastic condition.

25 - A method for determining the presence of the phTC protein in a cell or cell sample, which method is based on amplifying an hTC polynucleotide, or hybridizing an hTC polynucleotide, a primer or an hTC-complementary sequence with an hTC polynucleotide.

30 - A test kit for detecting phTC in cell samples and body fluids, with it being possible, for example, for labelled, immunochemically-reactive components to be: polyclonal

antibodies against phTC, monoclonal antibodies against phTC, fragments of these antibodies or a mixture of these components.

- 5 - A method for preventing and/or treating cell disturbance or destruction and/or malfunction and/or other symptoms in humans, which method is based on administering a therapeutically effective quantity of catalytically active human telomerase, its functional equivalents or its catalytically active fragments. It is also possible to conceive of using a substance which promotes the production and/or activity of phTC; a substance which can imitate the activity of phTC; a substance
- 10 which can inhibit the production and/or activity of phTC, or a mixture of these substances. A specific binding partner can also be employed.

The method is preferably employed for preventing or treating ageing or cancer diseases.

15 Substances which are able to affect the activity of phTC, i.e. inhibit or promote, are here termed modulators. Such modulators can be found, in a manner known per se, by testing their effect on telomerase activity in a telomerase assay. Examples of telomerase assays are given in Example 15.

20 Modulators of phTC are of interest for treating diseases which are connected with telomerase. The prevention or treatment of ageing processes or of cancer diseases may, in particular, be mentioned in this context.

- 25 - An antisense nucleic acid against the hTC mRNA, which nucleic acid comprises a nucleotide sequence which hybridizes with the said mRNA, with the antisense nucleic acid being an RNA or a DNA.

Preferably, the antisense nucleic acid binds to the start codon of the particular mRNA.

- A recombinant DNA molecule which contains a DNA sequence from which an antisense ribonucleic acid against the hTC mRNA is produced during transcription. This said antisense ribonucleic acid comprises a nucleic acid sequence which can hybridize to the said hTC mRNA.

5

A DNA molecule of this nature can be used to prepare a cell line having a reduced expression of pHTC by transfecting a pHTC-producing cell line with this recombinant DNA molecule.

10

- A ribozyme which cleaves the hTC mRNA.

This ribozyme is preferably a *Tetrahymena*-type ribozyme or a hammerhead-type ribozyme.

15

- A recombinant DNA molecule which contains a DNA sequence whose transcription leads to the production of a ribozyme of this nature.

This recombinant DNA molecule can be used to transfect a pHTC-producing cell line.

20

- A combination which consists of a pair of human hTC polynucleotide PCR primers, with the primers preferably consisting of sequences which correspond to the sequence of the human hTC mRNA or which are complementary to this sequence.

25

- A combination which comprises a polynucleotide hybridization probe for the human hTC gene, with the probe preferably comprising at least 29 consecutive nucleotides which correspond to the sequence of the human hTC gene or which are complementary to this sequence.

30

- Animal models which can be used to investigate telomerase/telomere regulation *in vivo*. Thus, tumour development and ageing can, for example, be directly investigated using knockout animals or transgenic animals.

In the case of proteins or peptides, functional equivalents are those compounds which, while being distinguishable with regard to amino acid sequence, essentially have the same functions.

5

Known examples of these compounds are isoenzymes or so-called microheterogeneities in proteins.

10

In the case of the oligonucleic or polynucleic acids, functional equivalents are to be understood as being those compounds which differ in nucleotide sequence but which encode the same protein. The existence of such compounds may be attributed, for example, to the fact that the genetic code is degenerate.

15

Explanation of the figures:

20

Fig. 1: cDNA sequence of the catalytic subunit of human telomerase (hTC) (SEQ ID No. 1).

Fig. 2: Amino acid sequence which is deduced from the hTC DNA sequence depicted in Fig. 1 (SEQ ID No. 2).

The DNA sequence depicted in Fig. 1 can be completely translated from Position 64 to Position 3461 into an amino acid sequence. The amino acid residues are depicted in accordance with their single-letter code.

25

Fig. 3: Ethidium bromide-stained agarose gel containing AA281296 DNA which has been treated in different ways.

30

The figure shows an ethidium bromide-stained 0.8% agarose gel. Two different DNA size standards are loaded in lanes 1 and 8, with the DNA fragment lengths 3, 2, 0.5 and 0.4 kb being pointed out. The AA281296 DNA in pT7T3D was digested with a restriction enzyme Eco RI/Not I (lane 3), Pst I (lane 6) and Xho I (lane 7). Undigested AA281296 DNA in pT7T3D was loaded onto lane 2. 1/10 of a PCR

mixture (1 minute 94°C, 2 minutes at 60°C, 3 minutes at 72°C) with the hTC cDNA in pT7T3D and primers 1 (5' GAGTGTGTACGTC-GTCGAGCTGCTCAGGTC 3') and 4 (5' CACCCTCGAGGTGAGACGCTCGGCC 3') [lane 4] and, especially, with primers 6 (5' GCTCGTAGTTGAGCACGCTGAACAGTG 3') and 7 (5' GCCAAGTTCCTGCACTGGCTGATGAG 3') [lane 5] was applied to lanes 4 and 5.

Fig. 4: Detail from a comparison of the protein sequences of the *Euplotes* p123 (p123) and human (phTC) catalytic telomerase subunits.

The conditions (ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison, using the Lasergene program software (Dnastar, Inc.), which is depicted in this figure. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between *Euplotes aediculatus* p123 and the identified EST₊₁ are also highlighted using the corresponding letter from the single-letter code. Amino acids which are not identical but whose function is similar or comparable are marked by a :.

Fig. 5: Part of a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* p123 (p123), and yeast (est2p).

The condition (Ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison using Lasergene program software (Dnastar, Inc.) which is depicted in this figure. The amino acid residues are shown in accordance with their single letter code. The amino acids which are identical between *Euplotes aediculatus* p123 and yeast est2p are likewise given prominence by the corresponding letter from the single-letter code. Amino acids which are not identical, but which are similar or comparable in function, are marked with a :.

Fig. 6: Detail from a comparison of the protein sequences of the yeast (est2p) and human (phTC) catalytic telomerase subunits.

The conditions (ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison, using the Lasergene program software (Dnastar, Inc.), which is depicted in this figure. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between yeast est2p and the identified EST₊₁ are also highlighted using the corresponding letter from the single-letter code. Amino acids which are not identical but whose function is similar or comparable are marked by a :.

Fig. 7: Detail from a comparison of the protein sequences of the *Euplotes* p123 (p123), yeast (est2p) and human (phTC) catalytic telomerase subunits. The comparison, depicted in Fig. 5, between *Euplotes* p123 (p123), yeast (est2p) and humans (phTC) was carried out using the Clustal Method subprogram of the Lasergene program software (Dnastar, Inc.) under standard conditions. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between yeast est2p, *Euplotes aediculatus* p123 and the identified EST₊₁ are also highlighted using the corresponding letter from the single-letter code. In addition, the regions which are identical between all three proteins are marked by a light grey bar above the protein sequence.

Fig. 8: Generated DNA sequence from Example 6 (RACE round 1) (SEQ ID No. 3).

Fig. 9: Generated DNA sequence from Example 6 (RACE round 2) (SEQ ID No. 4).

Fig. 10: Generated DNA sequence from Example 6 (RACE round 3) (SEQ ID No. 5).

Fig. 11: Generated DNA sequence from Example 8 (RACE round 3) (SEQ ID No. 6).

Fig. 12: Outline of the cloning of the complete hTC cDNA sequence. The positions of the start and stop codons are marked by arrows. The black regions of the rectangles symbolize protein-encoding sequence sections, whereas the pale grey regions symbolize 5'- and 3'- untranslated cDNA regions and/or denote intronsequences.

The dark grey blocks in the rectangle for the full-length cDNA either denote the telomerase-specific motif (T) or the seven reverse transcriptase motifs (numbers 1-7).

The DNA fragments which are required for preparing the complete hTC cDNA are likewise depicted as rectangles and are marked in accordance with their origin. All the rectangles are arranged in their positions relative to each other. The origin of the DNA fragment which is denoted by rectangle AA261296 is described in Example 2. The relative position of the 182 bp deletion in this fragment (compare Example 2) is shown by a gap in the rectangle. The origin of the DNA fragments which are denoted by the rectangles RACE 1, RACE 2 and RACE 3 is described in Example 6. The origin of the DNA fragment which is denoted by the C5F fragment rectangle is described in Example 7. The origin of the DNA fragment which is denoted by the lambda 12 rectangle is described in Example 9. The 3' part in the lambda 12 DNA fragment which encodes a cDNA which is not connected to hTC (compare Example 9) is not depicted in this figure. The complete hTC-cDNA sequence was joined together at the 5' and 3' splice sites using the lambda 12 and C5F DNA fragments shown in this figure (compare Example 7). These splice sites were identified in a variety of fragments (RACE 1, RACE 3, lambda 12 and C5F).

Fig. 13: Detailed sections from a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* and man (hTC).

The figure shows sections from a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* and man (hTC). Attention is drawn to the reverse transcriptase motifs in the boxed-in areas. The figures under the boxes refer to the respective amino acid positions in Fig. 2. The amino acid residues are shown in accordance with their single-letter code. Identical amino acids are printed in bold. In the consensus sequence for the reverse transcriptase (RT consensus) motif, h denotes a hydrophobic amino acid and p denotes a polar amino acid. If these groups of amino acids are retained in the *Euplotes* and hTC amino acid sequences, p and/or h is/are then printed in bold. Very highly conserved amino acids are underlaid in grey. In RT3, the boxed-in area is extended in order to cover

additional homologous amino acids. The telomerase-specific motif is described in Example 9.

Fig. 14: Generated DNA sequence from Example 11 (3' version) (SEQ ID No. 7). The region which is not homologous with the DNA sequence depicted in Fig. 1 is made to stand out in bold.

Fig. 15: hTC expression in cancer cell lines and normal human tissue. Fig. A: Approximately 2 μ g of poly-A⁺ RNA from different human cell lines were immobilized on the Northern blot in accordance with the manufacturer's (Clontech) instruction. Specifically, the RNA originated from a melanoma (G361), a lung carcinoma (A549), an adenocarcinoma of the colon (SW480), from a Raji Burkitt's lymphoma, from a leukaemia cell line (MOLT-4), from a chronic leukaemia cell line (K-562), from a cervical tumour (HeLa) and from the leukaemia cell line HL60. The transcripts marked 4.4 kb, 6 kb and 9.5 kb are specific for hTC (compare Example 10). Fig. B: About 2 μ g of poly-A⁺ RNA from different human tissues were immobilized on the Northern blot in accordance with the manufacturer's (Clontech) instructions. Specifically, the RNA was isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. An RNA size standard is shown.

Fig. 16: Western blot analysis of the rabbit sera against peptides from the human telomerase amino acid sequence (Example 12). In each case, 20 μ l of the bacterial lysates from Example 13 were analysed in a western blot (Ausubel *et al.*, 1987) using the antisera from Example 12. Lysates from bacteria which harbour the pMALEST construct were loaded in lanes 1, 2, 6 and 7. Lysates from bacteria which harbour the pMALA1 construct were loaded in lanes 3, 4, 8 and 9. Lysates from bacteria which were not induced with IPTG (isopropyl-beta-thiogalactopyranoside) were loaded in lanes 1, 3, 6 and 8. Lysates from IPTG-induced bacteria were loaded in lanes 2, 4, 7 and 9. A standard size marker (10 kDa protein ladder from Life Technologies, Cat. No. 10064-012) was loaded

in lane 5. The 50 kDa and 120 kDa bands are marked at the edges of the membranes. The PVDF membrane in Fig. A containing lanes 1 to 4 was incubated with preimmune sera against peptide B (compare Example 12). The PVDF membrane in Fig. B containing lanes 6 to 9 was incubated with preimmune sera against peptide C (compare Example 12). The PVDF membrane in Fig. B containing lanes 1 to 4 was incubated with immune sera against peptide B (compare Example 12). The PVDF membrane in Fig. B containing lanes 6 to 9 was incubated with immune sera against peptide C (compare Example 12).

Fig. 17: Autoradiogram of ^{35}S -labelled, *in vitro*-translated protein. The complete *in vitro*-translated hTC (compare Example 15) was loaded in lane 1. A C-terminally truncated version of phTC was loaded in lane 2. Lane 3 shows a positive control for the *in vitro* translation which was supplied by the manufacturer (compare Example 15). A protein size standard for estimating protein sizes is marked on the right-hand side.

Fig. 18: Autoradiogram of ^{32}P -labelled products from the TRAP assay (compare Example 15). A TRAP assay mixture without any added enzyme or protein was loaded, as a negative control, in lanes 1 and 2. A TRAP assay mixture containing partially purified human telomerase from HeLa cells was loaded, as a positive control, in lanes 3 and 4. A TRAP assay mixture containing *in vitro*-translated phTC was loaded, undiluted, in lanes 5 and 6. A TRAP assay mixture containing *in vitro*-translated phTC, at a 1:4 dilution, was loaded in lanes 7 and 8. A TRAP assay mixture containing *in vitro*-translated phTC, at a dilution of 1:16, was loaded in lanes 9 and 10. A TRAP assay mixture containing *in vitro*-translated luciferase was loaded, as a negative control, in lanes 11 and 12.

Fig. 19: Autoradiogram of ^{32}P -labelled products from the direct telomerase assay (compare Example 15). A radioactively labelled 10 bp marker was loaded in lane 1. A telomer oligonucleotide ($[\text{T TAGGG}]_3$) which was radioactively labelled 5' was loaded in lane 2. Lane 3 is an empty lane. Partially purified human telomerase

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from HeLa cells was used in a direct assay and the synthesis product was loaded, as a positive control, in lane 4. The *in vitro*-translated phTC from Example 15 was used in a direct assay and the synthesis product was loaded in lane 5.

Examples

Example 1

5 It is nowadays accepted that less than 5% of the human genome is in fact transcribed and translated into protein. Even before the genome has been completely sequenced, it is possible to obtain important information about the 60,000-70,000 genes in a human cell by investigating these coding moieties of the genome in a specific manner. The automation of high-throughput DNA sequencing technology in the last 10 to 15 years has made it possible
10 to collect many cDNAs from plasmid cDNA libraries of widely differing origin and sequence the 5' or 3' end in each case. These short DNA sequences, which are typically of from 300 to 400 bp in length, are termed expressed sequence tags or ESTs for short and are compiled in various specialized data bases. The EST approach was initially described by Okubo *et al.* (1992) and transferred to a larger scale by Adams *et al.* (1992). At present, approximately
15 50,000 human cell genes are partially sequenced and documented as EST entries.

By comparing with the DNA and amino acid sequences of known genes, it is possible to identify related, but hitherto unknown, genes in these EST databases (Gerhold and Caskey, 1996). tBLASTn (Altschul *et al.*, 1990) is a search algorithm which has proved particularly
20 useful for this purpose. This algorithm translates every DNA clone in the EST data base in all six possible reading frames and compares these amino acid sequences with the known protein sequence.

The EST data base at the National Center for Biotechnology Information (NCBI) was
25 searched with the recently published protein sequence for the *Euplotes aediculatus* catalytic telomerase subunit *p123* (Lingner *et al.*, 1997). This resulted in a human EST with the accession number AA281296 being identified which exhibits significant homology with p123 in reading frame +1. This amino acid sequence in reading frame +1 is termed Est₊₁ in that which follows.

The homology between p123 and the Est₁ is most conspicuous in two sequence regions which are separated by 30 amino acids. The longer sequence region, which in p123 extends from amino acid 438 to amino acid 484, is 38% identical to the corresponding region Est₁. If similar amino acids are also taken into consideration, the congruence is even 59%. The second block of homology extends, in the p123 protein, from amino acid 513 to amino acid 530 and exhibits 44% identity with the corresponding sequence segment in the identified Est₁. A congruence of 61% is obtained when amino acid residues having similar properties are taken into account.

The P (probability) value is an important parameter for assessing a BLAST search. P indicates the probability of also finding a specific segment pair in a BLAST search using a random sequence and varies numerically between 0 (highly significant result) and 1 (insignificant result). Thus, comparison of the p123 equivalent from yeast (est2p) with the NCBI EST data base, for example, gave a negative result: The EST which was found had a probability of P=1 (Tab. 1). On the other hand, human telomerase-associated protein 1 (hTP1), which was found in an EST data base which is not available to the general public (Harrington *et al.*, 1997), gives a probability of P=0.004.

known gene (species)	P	identified gene	origin of the cDNA library
est2p (<i>Saccharomyces cerevisiae</i>)	0.999	Rat EST	Kidney
p80 (<i>Tetrahymena termophila</i>)	0.004	hTP1 (Harrington <i>et al.</i> , 1997)	Crypts of the intestinal epithelium
p123 (<i>Euplotes aediculatus</i>)	$3.5 \cdot 10^{-06}$	AA281296	Germinal centres of the tonsils

Tab. 1: Comparison of three tBlastn search runs using different known genes.

The human EST AA281296 which was identified by the comparison with p123 has a probability of $P=3.5 \times 10^{-6}$.

5 These data suggest that the identified EST in all probability encodes a fragment of the catalytic subunit of human telomerase. For this reason, the corresponding gene is abbreviated below to hTC (human Telomerase, catalytic) and the deduced protein is abbreviated to phTC.

Example 2

10 The EST which was identified by the comparison with p123 was fed into the EST data base on 2 April 1997 and has not been published in any journal. According to information obtained from the National Center for Biotechnology Information, the cDNA library which contains this EST clone was prepared as follows:

15 After the mRNA had been prepared from the germinal centres of the tonsils, a cDNA synthesis was carried out and the double-stranded cDNA fragments were cloned in an orientated manner, using the Not I and Eco RI restriction enzyme cleavage sites, into the vector pT7T3D-Pac.

20 The 389 bp which had been fed into the EST database were sequenced using the -28m13 rev2 primer supplied by Amersham (DNA sequence, see Fig. 1 Position 1685 to 2073).

25 Lasergene program software (Dnastar Inc.) was used to translate the DNA sequence of EST AA281296 in accordance with the human genetic code. The resulting amino acid sequence (Est₊₁) corresponds to Position 542 to 670 in Fig. 2.

The deduced protein sequence of Est₊₁ is composed of 129 amino acids, including 27 basic, 11 acidic, 51 hydrophobic and 28 polar amino acid residues.

The EST (AA281296) which was identified in Example 1 was obtained commercially from Research Genetics, Inc. (Huntsville) in the form of a plasmid transformed into *E. coli* and analysed experimentally:

5 As shown in the ethidium bromide-stained agarose gel depicted in Fig. 3, a fragment from EST AA281296 of approximately 2.2 kb in size is liberated from the vector pT7T3D after
10 subjecting the prepared plasmid DNA to restriction digestion. With the aid of a polymerase chain reaction (PCR), which was carried out in parallel and which made use of specific internal primers, EST AA281296 was inspected: the lengths of the expected PCR products are 325 and 380 bp and are in agreement with the lengths of the fragments which were found
15 experimentally (cf. tracks 4 and 5 in Fig. 3). This therefore demonstrated that the *E. coli* clone supplied by Research Genetics, Int. (Huntsville) therefore harbours the identified EST as a plasmid.

20 After the DNA had been prepared, the 2176 bp of the insert in total were identified by means of double-strand sequencing. A comparison of the DNA sequences of clone AA281296 and of the C5F fragment (compare Example 7) showed that there was a 182 bp deletion (Positions 2352 to 2533, Fig. 1) and that the open reading frame is consequently displaced in this region. In summary, the DNA sequence of clone AA281296 is composed of the sequence
25 information shown in Fig. 1 (Positions 1685 to 2351 and Positions 2534 to 4042).

Example 3

25 The tBLASTn comparison only identifies the regions in which there is the greatest agreement between p123 and Est₁ (amino acids 438-530, in p123), whereas the intervening amino acids are not taken into account. A Lipman-Pearson protein comparison was carried out in order to be able to draw conclusions about the relatedness of the protein sequences over a larger region (amino acids 437-554, in p123) (see Fig. 4). When this was done, 34% of the amino acids were found to be identical while 59% of the amino acids were found to be either
30 identical or biochemically similar. This result demonstrates that the relatedness of these

proteins also continues outside the regions of homology which were found using the tBLASTn program.

As has recently been reported (Lingner *et al.*, 1997), *Euplotes aediculatus* p123 and *Saccharomyces cerevisiae* est2p are homologous to each other. In order to relate the degree of affinity between p123 and est2p to the homology between p123 and Est₊₁ which is described here, the Lipman-Pearson protein comparison was employed to compare the above-described region of p123 (amino acids 437-554) with est2p, too, using identical parameters. This showed that, in this chosen region, p123 and est2p are 21% identical and that 22% of their amino acid residues are either identical or biochemically similar (see Fig. 5). Accordingly, the homology between Est₊₁ and *Euplotes* p123 is significantly higher than between p123 and est2p.

Example 4

The homology of p123 with Est₊₁ and est2p suggests that all 3 proteins belong to the same protein family. In order to confirm this assumption, est2p was compared with Est₊₁ under the conditions described in Example 3 (see Fig. 6). This showed that Est₊₁ is 20% identical to est2p, that is exhibits a degree of homology which is comparable to that of p123 to est2p. This comparatively low level of congruence also confirms the finding that no significant EST was identified in the tBLASTn search using est2p (see Example 1).

Example 5

A computer comparison using p123, est2p and phTC was carried out in order to identify possibly functional domains which are important for the protein family consisting of catalytic telomerase subunits derived from different species (see Fig. 7). In this analysis, two regions which are present in all three proteins are particularly conspicuous (see Fig. 7). At present, no unambiguous function can be assigned to the region which, in p123, corresponds to amino acids 447 to 460 (Fig. 13, telomerase motif). A motif search using the Genetics Computer

Group (GCG) Wisconsin Sequence Analysis Package and a search in a protein data base (Swissprot, version of 8.6.1997) did not provide any significant insights.

On the other hand, a second region which is homologous between p123, est2p and phTC, corresponding in p123 to amino acids 512-526, exhibits a consensus motif for a reverse transcriptase (RT) (Figs. 7 and 13). Lingner *et al.*, (1997) showed that p123/est2p contain a total of 6 such RT motifs, which are essential for the catalytic function of p123/est2p. As depicted in Figs. 7 and 13, two such RT motifs are also conserved in the sequence of phTC which has been investigated. These motifs are the RT motifs which are located to the furthest extent N-terminally in p123/est2p (Lingner *et al.*, 1997).

The primary sequences of reverse transcriptases are strongly divergent; only a few amino acids are fully conserved within a separate motif (Poch *et al.*, 1989 and Xiong and Eickbush, 1990). In addition, due to having different distances between the conserved RT motifs, reverse transcriptases which are encoded by retroviruses or long terminal repeat (LTR) retroposons differ from those reverse transcriptases which are encoded by non-LTR retroposons or group II introns (Xiong and Eickbush, 1990). Based on the structure of their RT motifs, p123, est2p and phTC are to be assigned to the latter RT group. Interestingly, in this context, the consensus sequences of the RT motifs in phTC correspond most closely to the postulated RT consensus motif: of eight amino acid residues within the two RT motifs, 6 are present in the case of phTC while only 5 are present in the case of p123 and esp2p (Figs. 7 and 13). It is striking in this context how the hydrophobic amino acids, such as leucine and isoleucine, and the amino acids lysine and arginine, in particular, are in specific positions (Figs. 7 and 13).

In summary, it was hereby possible to demonstrate, at the descriptive level, that the AA281296 clone, identified due to its homology with p123, is a fragment of the catalytic subunit of human telomerase.

Example 6

For cloning the 5' end of the hTC-cDNA, three consecutive RACE (rapid amplification of cDNA ends) reactions were carried out in addition to the homology screening described in Example 8. Marathon-Ready cDNA (Clontech) from the human leukaemia cell line K562 or from human testis tissue was employed as the cDNA source. The implementation of the individual RACE rounds, as well as the results obtained, are described below.

In addition to this, the sequence information obtained in the RACE rounds was used in order to amplify the individual fragments from a contiguous cDNA clone by means of PCR.

RACE round 1:

In a final volume of 50 µl, 10 pmol of dNTP-mix were added to 5 µl of K562 Marathon-Ready cDNA (from Clontech, Catalogue Number 7441-1), and a PCR reaction was carried out in 1 × Klen Taq PCR reaction buffer and 1 × advantage Klen Taq polymerase mix (from Clontec). 10 pmol of the internal gene-specific primer GSP2 (5' -GCAACTTGCTCCAGACACTTCTTCCGG-3') from the 5' region of the hTC-EST clone and 10 pmol of the Marathon Adaptor primer AP1 (5'-CCATCCTAATACGACTCACTATAGGGC-3'; from Clontech) were added as primers. The PCR was carried out in 4 steps. After a one-minute denaturation at 94°C, denaturation was then carried out for 5 cycles of 30 sec at 94°C and the primers were then subsequently annealed for 4 min at 72°C and the DNA chain was extended. There then followed 5 cycles in which the DNA was denatured for 30 sec at 94°C but the subsequent primer extension took place for 4 min at 70°C. Finally, 22 cycles were then carried out in which, after the 30 sec DNA denaturation, the primer annealing and chain extension took place for 4 min at 68°C.

Following this PCR, the PCR product was diluted 1:50. 5 µl of this dilution were used in a second "nested" PCR together with 10 pmol of dNTP-mix in 1 × 10 Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix and also 10 pmol of primer GSP2 and

10 pmol of the "nested" Marathon Adaptor primer AP2 (5'-ACTCACTATAGGGCTCGAGCGGC-3'; from Clontech). The PCR conditions corresponded to the parameters selected in the first PCR. As the only exception, only 16 cycles were chosen, instead of 22 cycles, in the last PCR step.

5 A DNA fragment of 1153 bp in length was obtained as the product of this nested RACE PCR. This fragment was cloned into the TA cloning vector pCR2.1 from Invitrogen and subjected to complete double-strand sequencing (Fig. 8 and SEQ ID No. 3).

10 Nucleotides 974 to 1153 represent the nucleotide region 1629 to 1808 of the hTC-cDNA which is depicted in Fig. 1. The nucleotide region extending from bp 1 to bp 973, which does not exhibit any homology with the hTC-cDNA sequence shown in Fig. 1, represents intron sequences of the hTC gene (data not shown). A 3' splice consensus sequence is located at the exon-intron transition. The presence of intron sequences could be due to using incompletely
15 spliced mRNA as the starting substance for the cDNA synthesis. Genomic DNA contamination in the cDNA could also be an explanation for intron sequences being found.

RACE round 2:

20 Based on the sequence data obtained in the first RACE round, a second RACE was carried out using the gene-specific primer GSP5 from the 5' region of RACE product 1 (5'-GGCAGTGACCAGGAGGCAACGAGAGG-3') and the AP1 primer. Marathon-Ready cDNA from human testis (from Clontech; Catalogue Number 7414-1) was used as the cDNA source. The same PCR conditions were selected as in the 1st PCT in RACE round 1. The 1st
25 PCR was also followed, in RACE round 2, by a 2nd "nested" PCR using diluted PCR product as the cDNA source. The gene-specific primer GSP6 from the 5' region of RACE product 1 (5'-GGCACACTCGGCAGGAAACGCACATGG-3') and the AP2 primer were used as the "nested" PCR primers. The conditions corresponded to parameters for the nested PCR from RACE round 1.

The PCR product of 412 bp in length from the nested PCR of RACE round 2 was cloned into the TA cloning vector pCRII-Topo from Invitrogen and sequence completely (Fig. 9 and SEQ ID No. 4). The sequence segment from bp 267 to bp 412 is completely homologous with the 5' region of the product from RACE 1. The region from bp 1 to bp 266 extends RACE product 1 at the 5' end. This RACE product 2 is probably, in its entirety, an intron region of the hTC gene (data not shown).

RACE round 3:

A third RACE round led to the identification of hTC-cDNA regions which were located further on in the 5' direction. Using the sequence results from RACE round 2 as a base, a gene-specific primer GSP9 (5'-CCTCCTCTGTTCAGTCTGGCC-3') was selected from the 5' region of RACE product 2 and used in a new RACE together with the AP1 primer and Marathon-Ready cDNA from human testis (from Clontech). The RACE conditions were the same as those used in the 1st PCR in RACEs 1 and 2. In the "nested" RACE which followed, and which took place, in accordance with the "nested" RACEs in rounds 1 and 2, using the gene-specific primer GSP10 from the 5' region of RACE product 2 (5'-CGTAAGTTTATGCAAAGTGGACAGG-3') and AP2, a fragment of 1012 bp in length (Fig. 10 and SEQ ID No. 5) was amplified and cloned into the TA cloning vector pCRII-TOPO. Subsequent sequencing showed that the 3' region of this RACE fragment (bp 817 - bp 1012) evidently still constitutes an intron sequence of the hTC gene. The region from bp 889 to bp 1012 is completely homologous with the 5' region of RACE product 2. On the other hand, the 5' region of this fragment, from bp 1 to bp 816, is identical to the bp 814 - bp 1629 region of the hTC-cDNA which is shown in Fig. 1. A potential 5' splice consensus sequence is located at the exon-intron transition.

Example 7

A PCR was carried out in order to clone a contiguous fragment from the sequence information obtained from RACE 2 and clone AA281296. Marathon-Ready cDNA from human testis (from Clontech; Catalogue Number 7414-1) was used as the cDNA source. The

PCR mixture was as described under RACE 1 (compare Example 6) but using the primers C5F (5'-CGAGTGGACACGGTGATCTCTGCC-3') from the 5' region of RACE 2 and primer C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3') from a 3' region of clone AA281296. The PCR was carried out in 2 steps. After a one-minute denaturation at 94°C, denaturation was then carried out for 36 cycles of 30 sec at 94°C and, after that, the primers were annealed, and the DNA chain was extended, for 4 min at 68°C.

A DNA fragment of 2486 bp in length, which is designated the C5F fragment below, was obtained as the product of this PCR. This fragment was cloned into the TA cloning vector pCRII-TOPO from Invitrogen and subjected to complete double-strand sequencing. A comparison of the DNA sequences of the C5F fragment and the AA281296 clone showed that there was an in-frame insertion of 182 bp between RT motif 3 and RT motif 4 (Positions 2352 to 2533, Fig. 1). A further comparison of DNA of the C5F fragment with the sequences from the three RACE rounds made it clear that an intron which was already identified in RACE 2 was present at the 3' end of C5F. A 3' splice consensus sequence is located at the exon-intron transition. In summary, the DNA sequence of the C5F fragment is consequently composed of the sequence information shown in Fig. 9 (Position 64 to 278) and the sequence data shown in Fig. 1 (Positions 1636 to 3908).

Example 8

For cloning the 5' end of the hTC-cDNA, a homology screening (Ausubel *et al.*, 1987) was carried out in addition to the RACE protocol described in Example 6. A human erythroleukaemia 5'-stretch plus cDNA library (from Clontech, cat. No. HL5016b) from the human leukaemia cell line K562 was used as the cDNA source. Approximately 3×10^6 Pfu of this random and oligo-dT-primed library were plated out and used for screening as described in Ausubel *et al.* (1987). A radioactively labelled hTC-DNA fragment of 719 bp in length (Positions 1685 to 2404, corresponding to Fig. 1) was used as the probe.

Following a rescreening with the same hTC probe, the λ clone 12 was verified as being positive out of 20 putatively positive λ clones. Following plaque purification and λ DNA

preparation (Ausubel *et al.*, 1987), the 4 kb insert was recloned into the pBluescript vector and sequenced (Fig. 11 and SEQ ID No. 6).

A comparison of the λ clone 12 sequence with the sequences of the RACE clones and the DNA sequence of clone AA281296 showed that this clone, which was identified in the homology screening, encodes a 5' part of the hTC-cDNA and possesses a putative ATG start codon in Position 63 in accordance with Fig. 1. There is no stop codon in the same reading frame 5' of this ATG. Subsequent sequence analyses make it clear that λ clone 12 probably contains an intron from Positions 1656 to 2004. Very well conserved 5' and 3' splice sites provide support for this hypothesis. The hTC-cDNA-encoding sequence then continues from Position 2005 to Position 2382. The sequence from 2383 to the 3' end of λ clone 12 exhibits a conspicuous open reading frame in reading frame -4. A bioinformatic analysis of the corresponding DNA sequence showed that, over about 400 bp, this reading frame is identical to a variety of ESTs which have no connection with the hTC cDNA. Consequently, λ clone 12 is a chimeric clone which essentially consists of the 5' end of the hTC cDNA and another cDNA clone of unknown function.

A diagrammatic summary showing the relative orientations of the RACE products, and the homology screening, is depicted in Fig.12. The complete sequence of the hTC cDNA (Fig. 1) was assembled from λ clone 12 (Positions 21 to 1655 in accordance with Fig. 11), the C5F PCR product (Positions 1636 to 3908 in accordance with Fig. 1) and EST AA281296 (Positions 3909 to 4042, in accordance with Fig. 1).

Example 9

A total of seven motifs for reverse transcriptases (RT motifs) was identified by comparing the phTG protein sequence (Fig. 2 and SEQ ID NO. 2) with a reverse transcriptase consensus sequence (Poch *et al.*, 1989, Xiong and Eickbush, 1990) (Fig. 13). Within these motifs, some amino acids are highly conserved not only between the RT consensus sequence and phTC but also in comparison with the *Euplotes* telomerase protein. Thus, two aspartic acids (Positions 868 and 869 in Fig. 2) are, for example, completely conserved in RT motif 5 (Fig. 13). RT

motif 7, which was deduced from other reverse transcriptases (Poch *et al.*, 1989, Xiong and Eickbush, 1990), was only demonstrated in the human catalytic telomerase subunit and not in the *Euplotes* protein (Fig. 13).

5 Structural features which can only be found in the telomerase proteins and not in other reverse transcriptases are also conspicuous. The telomerase motif (Positions 553 and 565 in Fig. 2) is a structure which is specific for this protein family since it does not occur in any previously known protein. A further feature which has only been identified in the catalytic telomerase proteins is the difference between RT motifs 3 and 4, which distance, at
10 107 amino acids, is markedly greater than in other RTs. These special features indicate that the catalytic subunits of the telomerases from different species probably constitute a separate subgroup of RNA-dependent DNA polymerases.

Example 10

15 Expression of the telomerase RNA subunit (hTR) does not correlate with telomerase activity but, instead, is observed ubiquitously (Feng *et al.*, 1995). Consequently, the question arises as to whether expression of the catalytic telomerase subunit is associated with telomerase activity.

20 Northern blot experiments (Ausubel *et al.*, 1987) were carried out in order to analyze the level of hTR expression. The commercially available Northern blots were supplied with a number of RNA preparations from normal human tissue (from Clontech; catalogue No. 7760-1) or with RNA samples from human cancer cell lines (from Clontech; Catalogue
25 Number 7757-1). A radioactively labelled hTR DNA fragment of 719 bp in length (Positions 1685 to 2404, in accordance with Fig. 1) was used as the probe. The membranes were incubated with the probe in accordance with the manufacturer's (Clontech) instructions.

30 Two main RNA transcripts, of about 9.5 kb and 4.4 kb in size, and an additional RNA transcript of about 6 kb, which transcripts cross-hybridize with the probe, were detected in the eight human cell lines (3 leukaemia cell lines, 3 carcinoma cell lines, one melanoma and one

lymphoma) tested (Fig.15, Fig. A). In the comparison, the hTC mRNA was expressed most strongly in the leukaemia cell lines K-562 and HL-60 (Fig. 15, Fig. A). By contrast, it was not possible to detect the hTC transcript in the normal tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) which were tested (Fig. 15, Fig. B). This observation is not surprising since it was not possible to detect any telomerase activity, either, in these tissues (Kim *et al.*, 1994).

These data indicate that the induction of hTC expression plays an important role in activating the telomerase during tumour development.

Example 11

Several PCR products, whose sizes only differed from each other to a minimal extent, were always obtained when the hTC cDNA fragments from various cDNA libraries (Clontech Marathon Ready cDNA from the human leukaemia cell line K562 and from human testis and also cDNA from the human premyeloid leukaemia cell line HL60) were subjected to PCR amplification. In order to elucidate the differences between the different hTC-PCR products, a fragment of the hTC cDNA depicted in Fig. 1 extending from bp 1783 to bp 3901 was amplified using the primers C5A (5'-CCGGAAGAGTGTCTGGAGCAAGTTGC-3') and C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3'). Marathon-Ready cDNA from K562 leukaemia cells (from Clontech; Catalogue Number 7441-1) was used as the cDNA source (PCR1 and 2). In a third PCR, a hTC fragment, from bp 1695 to bp 3463, of the hTC cDNA in Fig.1 was amplified from HL60 cDNA using the primers GSP1 front (5'-GGCTGATGAGTGTGTACGTCGTCGAG-3') and HTRT3A (5'-GGGTGGCCATCAGTCCAGGATGG-3').

The conditions of the 3 PCR reactions are described below:

In the first PCR, and in a final volume of 50 µl, 10 pmol of dNTP mix were added to 5 µl of K562 Marathon-Ready cDNA, and a PCR reaction was carried out in 1 × Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix (from Clontech). 10 pmol of

each of the primers C5A and C5B were added. The PCR was carried out in 3 steps. A one-minute denaturation at 94°C was followed by 35 PCR cycles in which the DNA was firstly denatured for 30 sec at 94°C and the primers were then annealed, and the DNA chain was extended, for 4 min at 68°C. In conclusion, there followed a chain extension for 10 min at 68°C. The resulting PCR products were cloned into the TA cloning vector pCRII-TOPO from Invitrogen.

In a second PCR, 10 pmol of each of the primers C5A and C3B, 10 pmol of dNTP mix and 2 U of Taq DNA polymerase (from Gibco-BRL) were added to 5 µl of K562 Marathon-Ready cDNA, and a PCR reaction was carried out in 1 × PCR buffer (from Perkin Elmer) in a final volume of 50 µl. The PCR reaction was carried out in 3 steps. The DNA was firstly denatured for 3 min at 94°C. There then followed 34 cycles in which, consecutively, the DNA was denatured for 45 sec at 94°C, primer annealing then took place for 1 min at 68°C and, after that, the DNA chain was extended for 3 min at 72°C. In the last PCR step, a concluding chain extension was carried out for 10 min at 72°C. The resulting PCR products were cloned into the TA cloning vector pCR2.1 from Invitrogen.

For the third PCR, the cDNA synthesis kit from Boehringer Mannheim was first of all used to carry out a cDNA synthesis from 2 µg of DNaseI-treated poly-A RNA from the human premyeloid cell line HL60 in accordance with the manufacturer's instructions. 1 µl of this HL60 cDNA was then mixed with 10 pmol of each of the primers GSP1 front and HTRT3A and also 10 pmol of dNTP mix, in a final volume of 50 µl, and, after 1.25 µl of DMSO in 1 × Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix (from Clontech) had been added, a PCR reaction was carried out. The PCR reaction proceeded in 3 steps. After a denaturation for 3 min at 94°C, the DNA was initially denatured for 1 min at 94°C and the primers were then annealed, and the DNA chain extended, for 4 min at 68°C, over 37 cycles. The reaction was concluded by a further incubation for 10 min at 68°C. The PCR products were cloned into the TA cloning vector pCR2.1-TOPO.

Complete double-strand sequencing of the cloned hTC cDNA fragments from PCRs 1 and 2, and partial sequencing of the hTC cDNA fragments obtained from PCR 3, showed that, in addition to the hTC cDNA depicted in Fig. 1, 4 variants of this cDNA exist in human cells, i.e.:

Variant 1 of human hTC cDNA is distinguished by a deletion of 182 bp in length extending from nucleotides 2345 to 2526. This deletion results in the ORF being displaced, with a truncated hTC protein, which lacks RT motifs 4 to 7, being read off.

Variant 2 of human hTC cDNA exhibits a deletion of 36 bp in length extending from nucleotides 2184 to 2219. RT motif 3 is lost as a result of this deletion. However, the reading frame is retained and a protein is produced which selectively lacks RT motif 3.

Variant 3 of human hTC cDNA is a combination of variants 1 and 2. It exhibits both a deletion from bp 2184 to 2219 and a deletion from bp 2345 to 2526.

Variant 4 of human hTC cDNA is distinguished by the loss of the nucleotide region from bp 3219 to bp 3842. This missing sequence is replaced by a sequence which is not homologous with hTC. From bp 3843 onwards, the sequence is once again completely identical to the hTC sequence depicted in Fig. 1. The sequence of variant 4 is shown in Fig. 14. In accordance with the 5' primer chosen, it begins with bp 1783 of the hTC cDNA shown in Fig. 1. The region which is not homologous is emphasized in bold and, from Position 3219 to Position 3451 (Fig. 14 and SEQ ID No. 7) is, to the extent of 98.7%, in agreement, at the DNA level, with an EST (Accession No. AA299878) from a human uterus tumour.

Example 12

In order to obtain antisera having specificity for the catalytic subunit of human telomerase, the available nucleotide sequence (Fig. 1) was translated into an amino acid sequence (Fig. 2). Using a secondary structure prediction program (PROTEAN, from the DNASTar

software package, DNASTAR Inc., Madison, WI, USA), two peptides were chosen which, with a certain degree of probability, evoke an immune response. These are the following peptides, which are depicted in the one-letter code for amino acids:

- 5 B: C-K-R-V-Q-L-R-E-L-S-E-A-E-V-R-Q - CONH₂/Pos. 594 - 608
 C: C-Q-E-T-S-P-L-R-D-A-V-V-I-E-Q-S-S-S-L-N-E - CONH₂/Pos. 781-800

The cysteines which are underlined are not derived from the telomerase sequence but were additionally added on as linkers for the coupling.

10

The peptides were coupled to keyhole limpet hemocyanin (KLH) using the thiol-reactive coupling reagent m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS). Two rabbits were in each case immunized with these coupled peptides at intervals of from 2 to 4 weeks. Prior to immunization, 5 ml of blood were withdrawn in order to obtain preimmune sera. After 4 immunizations, 5 ml of blood were likewise withdrawn for obtaining immune sera. These sera were tested for reactivity with fusion proteins (Example 13) in a Western blotting experiment (Ausubel *et al.*, 1987).

15

Example 13

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Bacterial expression experiments were carried out in order to be able to analyse the protein of the catalytic telomerase subunit.

The constructs of these experiments are described below:

25

For the expression construct pMalEST, the insert in the AA281296 clone mentioned in Example 2 was excised with restriction enzymes Eco RI and Not I and the cleavage sites were filled in using the Klenow fragment (Ausubel *et al.*, 1987); the insert was then cloned into the given reading frame of the maltose-binding protein of the bacterial expression vector pMAL-C2 (from New England Biolabs). Vector pMAL-C2 was digested with restriction

30

enzyme Pst I and the protruding single-strand ends were removed with T4 DNA polymerase (Ausubel *et al.*, 1987).

5 The expression construct pMalA1 contains the nucleotide sequence of Fig. 1 from Position 1789 to Position 3908. This DNA fragment was amplified from a commercially available K562 Marathon-Ready cDNA library (from Clontech, Catalogue Number 7441-1) by means of PCR using the primers C5A (5'-ACCGGAAGAGTGTCTGGAGCAAGTTG-3') and C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3'), and cloned into the TA cloning vector pCRII-TOPO from Invitrogen. The PCR conditions were as described in Example 7.
10 For the expression construct pMalA1, the insert was excised using the restriction enzyme Eco RI and the cleavage sites were filled in using the Klenow fragment (Ausubel *et al.*, 1987); the insert was then cloned into the bacterial expression vector pMAL-C2 (from New England Biolabs) which had been cleaved with the restriction enzyme Xmn I.

15 These constructs were then used for protein expression in the bacterial strain *E. coli* DH5 α . The expression conditions were those as described in the instructions provided by New England Biolabs (Catalogue Number 800). The bacterial lysates which were prepared were tested in a Western blotting experiment (Ausubel *et al.*, 1987).

20 Example 14

The bacterial lysates from Example 13 were analysed in a Western blot (Ausubel *et al.*, 1987) using the antisera from Example 12.

25 Since the proportion of the fusion represented by the maltose-binding protein is about 43 kDa in size, fusion proteins of about 74 kDa and 106 kDa are expected for the pMalEST and pMalA1 constructs, respectively.

30 When comparing the preimmune sera with the sera following the first immunization, it becomes evident that specific antibodies were formed against the B and C epitopes (Fig. 16). Furthermore, in addition to the expected 74 kDa and 106 kDa proteins, respectively, smaller

protein fragments were also observed which react with the antisera. These smaller products probably originate from premature products.

Only the epitope for serum B is present on the fusion protein from the expression using pMalEST. By contrast, the epitopes for sera B and C are present on the fusion protein from pMalA1. For this reason, antiserum C does not recognize the pMalEST expression product and only recognizes the larger protein fragments from the expression experiments using pMalA1. This observation underlines the high degree of specificity of the antisera which were generated.

Example 15

In order to be able to analyse the protein of the catalytic telomerase subunit, the protein component should be reconstituted *in vitro* together with the RNA component.

The constructs for these experiments are described below:

The RNA component of 504 nt in length (Feng *et al.*, 1995) was amplified from a 293 cell cDNA library using the primers HTR9BAM (5'-CGCGG-ATCCTAATACGACTCACTATAGGGTTGCGGAGGGTGGGCCTG-3') and HTR2BAM (5'-CGCGGATCCCGGCGAGGGGTGACGGATGC-3'). Primer HTR9BAM contains a T7 promoter from nucleotide 10 to 29. In the PCR, 10 pmol of dNTP mix were added, in a final volume of 100 µl, to 3 µl of cDNA from 293 cells, and a PCR reaction was carried out in 1 × PCR reaction buffer containing 0.5 µl of Taq polymerase (from Gibco). 10 pmol of each of the primers HTR9BAM and HTR2BAM were added. The PCR was carried out in 3 steps. A ten-minute denaturation at 94°C was followed by 35 PCR cycles in which the DNA was first of all denatured for one minute at 94°C and, after that, the primers were annealed, and the DNA chain was extended, for 2 min at 62°C. In conclusion, there followed a chain extension for 4 min at 72°C. The resulting PCR products were cloned, after a restriction digestion with Bam HI, into the Bam HI cleavage site of vector pUC19 in such a way that the

RNA component is under the control of the T7 promoter. This construct is designated HTR504 in that which follows.

5 The cDNA fragment of 3411 bp in length (Position 60 to Position 3470, Fig. 1) was cloned into the vector PCRII TOPO (from Invitrogen). Detailed information on the cloning is given in Examples 8 and 7, and also in Fig. 12. In this construct, which is designated HTC FL, the T7 promoter is located 5' before the hTC cDNA.

10 The catalytic telomerase protein component was synthesized in a commercially available transcription/translation system, after adding the hTC FL construct, in accordance with the manufacturer's (Promega; Catalogue Number L4610) instructions. Whether the *in vitro* translation of the expected 127 kDa product had been successful was checked in an SDS-PAGE (Ausubel *et al.*, 1987) using ³⁵S-labelled cysteine (Fig. 17).

15 The telomerase RNA component was synthesized using a transcription system in accordance with the manufacturer's (Ambion; Catalogue Number 1344) instructions or using the method described by Pokrovskaya and Gurevich (1994).

20 For the *in vitro* re-constitution, 0.5 µg of hTRNA was added to 50 µl of the above-described translation mixture containing the hTC FL construct and the whole was incubated at 37°C for 10 min. The enzymatic activity of 2 µl of this mixture was investigated using the TRAP assay (N.W. Kim *et al.*, 1994). The measurement of the activity, by the same method, of telomerase which was purified from HeLa cells (Shay *et al.*, 1994) was used as the positive control. As
25 can be seen in Fig. 18, both the reconstituted enzyme and the native enzyme produce the same product pattern, i.e. the nucleotide ladder which is characteristic for telomerase. This result also verifies that a single protein component, together with the RNA, is sufficient for the enzymatic telomerase activity.

30 In addition to the described TRAP assay, 5 µl of the reconstitution mixture were tested for its activity in a direct telomerase assay (Shay *et al.*, 1994). In this experiment, too, the

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(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER-READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30B (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4042 Basenpaare
- (B) TYPE: Nucleotide
- (C) STRANDEDNESS: Einzelstrang
- (D) TOPOLOGY: Linear

(ii) ART DES MOLEKÜLS: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC CCTGGCCCCG GCCACCCCCG	60
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AGGTGCTGCC GCTGGCCACG TTCGTGCGGC GCCTGGGGCC CCAGGGCTGG CGGCTGGTGC	180
AGCGCGGGGA CCCGGCGGCT TTCCGCGCGC TGGTGGCCCA GTGCCTGGTG TGCCTGCCCT	240
GGGACGCACG GCCGCCCCC GCCGCCCCCT CCTTCCGCCA GGTGTCCTGC CTGAAGGAGC	300
TGGTGGCCCC AGTGCTGCAG AGGCTGTGCG AGCGCGGCGC GAAGAACGTG CTGGCCTTCG	360
GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCGCCCCCGA GGCCTTCACC ACCAGCGTGC	420
GCAGCTACCT GCCCAACACG GTGACCGACG CACTGCGGGG GAGCGGGGCG TGGGGGCTGC	480

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CTGCCACTCA	GGCCCGGCCC	CCGCCACACG	CTAGTGGAAC	CCGAAGGCGT	CTGGGATGCG	660
AACGGGCTTG	GAACCATAGC	GTCAGGGAGG	CCGGGGTCCC	CCTGGGCCTG	CCAGCCCCGG	720
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TGCGCAGGAG	CCCAGGGGTT	GGCTGTGTTT	CGGCCGCAGA	GCACCGTCTG	CGTGAGGAGA	1680
TCCTGGCCAA	GTTCTGCAC	TGGCTGATGA	GTGTGTACGT	CGTCGAGCTG	CTCAGGTCTT	1740
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GACTCCGCTT	CATCCCCAAG	CCTGACGGGC	TGCGGCCGAT	TGTGAACATG	GACTACGTCTG	1980
TGGGAGCCAG	AACGTTCCGC	AGAGAAAAGA	GGGCCGAGCG	TCTCACCTCG	AGGGTGAAGG	2040
CACTGTTTCA	CGTGCTCAAC	TACGAGCGGG	CGCGGCGCCC	CGGCCTCCTG	GGCGCCTCTG	2100
TGCTGGGCCT	GGACGATATC	CACAGGGCCT	GGCGCACCTT	CGTGCTGCGT	GTGCGGGCCC	2160
AGGACCCGCC	GCCTGAGCTG	TACTTTGTCA	AGGTGGATGT	GACGGGCGCG	TACGACACCA	2220
TCCCCCAGGA	CAGGCTCACG	GAGGTCATCG	CCAGCATCAT	CAAACCCAG	AACACGTACT	2280
GCGTGCGTCG	GATGCGCTG	GTCCAGAAGG	CCGCCCATGG	GCACGTCCGC	AAGGCCTTCA	2340

AGAGCCACGT CTCTACCTTG ACAGACCTCC AGCCGTACAT GCGACAGTTC GTGGCTCACC	2400
TGCAGGAGAC CAGCCCGCTG AGGGATGCCG TCGTCATCGA GCAGAGCTCC TCCCTGAATG	2460
AGGCCAGCAG TGGCCTCTTC GACGTCTTCC TACGCTTCAT GTGCCACCAC GCCGTGCGCA	2520
TCAGGGGCAA GTCCTACGTC CAGTGCCAGG GGATCCCGCA GGGCTCCATC CTCTCCACGC	2580
TGCTCTGCAG CCTGTGCTAC GCGGACATGG AGAACAAGCT GTTTGCGGGG ATTCGGCGGG	2640
ACGGGCTGCT CCTGCGTTTG GTGGATGATT TCTTGTTGGT GACACCTCAC CTCACCCACG	2700
CGAAAACCTT CCTCAGGACC CTGGTCCGAG GTGTCCCTGA GTATGGCTGC GTGGTGAAC	2760
TGCGGAAGAC AGTGGTGAAC TTCCCTGTAG AAGACGAGGC CCTGGGTGGC ACGGCTTTTG	2820
TTCAGATGCC GGCCACGGC CTATTCCCCT GGTGCGGCCT GCTGCTGGAT ACCCGGACCC	2880
TGGAGGTGCA GAGCGACTAC TCCAGCTATG CCCGGACCTC CATCAGAGCC AGTCTCACCT	2940
TCAACCGCGG CTTCAAGGCT GGGAGGAACA TGCCTCGCAA ACTCTTTGGG GTCTTGCGGC	3000
TGAAGTGTC CAGCCTGTTT CTGGATTGTC AGGTGAACAG CCTCCAGACG GTGTGCACCA	3060
ACATCTACAA GATCCTCCTG CTGCAGGCGT ACAGGTTTCA CGCATGTGTG CTGCAGCTCC	3120
CATTTTCATCA GCAAGTTTGG AAGAACCCCA CATTTTTCCT GCGCGTCATC TCTGACACGG	3180
CCTCCCTCTG CTACTCCATC CTGAAAGCCA AGAACGCAGG GATGTGCTG GGGGCCAAGG	3240
GCGCCGCCGG CCCTCTGCCC TCCGAGGCCG TGCACTGGCT GTGCCACCAA GCATTCTGTC	3300
TCAAGCTGAC TCGACACCGT GTCACCTACG TGCCACTCCT GGGGTCACTC AGGACAGCCC	3360
AGACGCAGCT GAGTCGGAAG CTCCCGGGGA CGACGCTGAC TGCCCTGGAG GCCGCAGCCA	3420
ACCCGGCACT GCCCTCAGAC TTCAAGACCA TCCTGGACTG ATGGCCACCC GCCCACAGCC	3480
AGGCCGAGAG CAGACACCAG CAGCCCTGTC ACGCCGGGCT CTACGTCCCA GGGAGGGAGG	3540
GGCGGCCAC ACCCAGGCC GCACCGCTGG GAGTCTGAGG CCTGAGTGAG TGTTTGCCG	3600
AGGCCTGCAT GTCCGGCTGA AGGCTGAGTG TCCGGCTGAG GCCTGAGCGA GTGTCCAGCC	3660
AAGGGCTGAG TGTCCAGCAC ACCTGCCGTC TTCCTTCCC CACAGGCTGG CGCTCGGCTC	3720
CACCCAGGG CCAGCTTTTC CTCACCAGGA GCCCGGCTTC CACTCCCCAC ATAGGAATAG	3780
TCCATCCCCA GATTGCCAT TGTTCACCCC TCGCCCTGCC CTCCTTTGCC TTCCACCCCC	3840
ACCATCCAGG TGGAGACCCT GAGAAGGACC CTGGGAGCTC TGGGAATTTG GAGTGACCAA	3900
AGGTGTGCCC TGTACACAGG CGAGGACCCT GCACCTGGAT GGGGGTCCCT GTGGGTCAAA	3960
TTGGGGGGAG GTGCTGTGGG AGTAAAATAC TGAATATATG AGTTTTTCAG TTTTGAAAAA	4020
AAAAAAAAAA AAAAAAAAAA AA	4042

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 1132 amino acids
 (B) TYPE: Amino acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(ii) ART DES MOLEKŠLS: Protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Pro	Arg	Ala	Pro	Arg	Cys	Arg	Ala	Val	Arg	Ser	Leu	Leu	Arg	Ser	1	5	10	15
His	Tyr	Arg	Glu	Val	Leu	Pro	Leu	Ala	Thr	Phe	Val	Arg	Arg	Leu	Gly	20	25	30	
Pro	Gln	Gly	Trp	Arg	Leu	Val	Gln	Arg	Gly	Asp	Pro	Ala	Ala	Phe	Arg	35	40	45	
Ala	Leu	Val	Ala	Gln	Cys	Leu	Val	Cys	Val	Pro	Trp	Asp	Ala	Arg	Pro	50	55	60	
Pro	Pro	Ala	Ala	Pro	Ser	Phe	Arg	Gln	Val	Ser	Cys	Leu	Lys	Glu	Leu	65	70	75	
Val	Ala	Arg	Val	Leu	Gln	Arg	Leu	Cys	Glu	Arg	Gly	Ala	Lys	Asn	Val	85	90	95	
Leu	Ala	Phe	Gly	Phe	Ala	Leu	Leu	Asp	Gly	Ala	Arg	Gly	Gly	Pro	Pro	100	105	110	
Glu	Ala	Phe	Thr	Thr	Ser	Val	Arg	Ser	Tyr	Leu	Pro	Asn	Thr	Val	Thr	115	120	125	
Asp	Ala	Leu	Arg	Gly	Ser	Gly	Ala	Trp	Gly	Leu	Leu	Leu	Arg	Arg	Val	130	135	140	
Gly	Asp	Asp	Val	Leu	Val	His	Leu	Leu	Ala	Arg	Cys	Ala	Leu	Phe	Val	145	150	155	
Leu	Val	Ala	Pro	Ser	Cys	Ala	Tyr	Gln	Val	Cys	Gly	Pro	Pro	Leu	Tyr	165	170	175	
Gln	Leu	Gly	Ala	Ala	Thr	Gln	Ala	Arg	Pro	Pro	Pro	His	Ala	Ser	Gly	180	185	190	
Pro	Arg	Arg	Arg	Leu	Gly	Cys	Glu	Arg	Ala	Trp	Asn	His	Ser	Val	Arg	195	200	205	
Glu	Ala	Gly	Val	Pro	Leu	Gly	Leu	Pro	Ala	Pro	Gly	Ala	Arg	Arg	Arg	210	215	220	
Gly	Gly	Ser	Ala	Ser	Arg	Ser	Leu	Pro	Leu	Pro	Lys	Arg	Pro	Arg	Arg	225	230	235	
																		240	

Gly	Ala	Ala	Pro	Glu	Pro	Glu	Arg	Thr	Pro	Val	Gly	Gln	Gly	Ser	Trp
				245					250					255	
Ala	His	Pro	Gly	Arg	Thr	Arg	Gly	Pro	Ser	Asp	Arg	Gly	Phe	Cys	Val
			260					265					270		
Val	Ser	Pro	Ala	Arg	Pro	Ala	Glu	Glu	Ala	Thr	Ser	Leu	Glu	Gly	Ala
		275					280					285			
Leu	Ser	Gly	Thr	Arg	His	Ser	His	Pro	Ser	Val	Gly	Arg	Gln	His	His
	290					295					300				
Ala	Gly	Pro	Pro	Ser	Thr	Ser	Arg	Pro	Pro	Arg	Pro	Trp	Asp	Thr	Pro
305					310					315					320
Cys	Pro	Pro	Val	Tyr	Ala	Glu	Thr	Lys	His	Phe	Leu	Tyr	Ser	Ser	Gly
			325						330					335	
Asp	Lys	Glu	Gln	Leu	Arg	Pro	Ser	Phe	Leu	Leu	Ser	Ser	Leu	Arg	Pro
		340						345					350		
Ser	Leu	Thr	Gly	Ala	Arg	Arg	Leu	Val	Glu	Thr	Ile	Phe	Leu	Gly	Ser
	355						360					365			
Arg	Pro	Trp	Met	Pro	Gly	Thr	Pro	Arg	Arg	Leu	Pro	Arg	Leu	Pro	Gln
	370					375					380				
Arg	Tyr	Trp	Gln	Met	Arg	Pro	Leu	Phe	Leu	Glu	Leu	Leu	Gly	Asn	His
385				390						395					400
Ala	Gln	Cys	Pro	Tyr	Gly	Val	Leu	Leu	Lys	Thr	His	Cys	Pro	Leu	Arg
			405						410					415	
Ala	Ala	Val	Thr	Pro	Ala	Ala	Gly	Val	Cys	Ala	Arg	Glu	Lys	Pro	Gln
		420						425					430		
Gly	Ser	Val	Ala	Ala	Pro	Glu	Glu	Glu	Asp	Thr	Asp	Pro	Arg	Arg	Leu
		435					440					445			
Val	Gln	Leu	Leu	Arg	Gln	His	Ser	Ser	Pro	Trp	Gln	Val	Tyr	Gly	Phe
	450					455					460				
Val	Arg	Ala	Cys	Leu	Arg	Arg	Leu	Val	Pro	Pro	Gly	Leu	Trp	Gly	Ser
465				470					475						480
Arg	His	Asn	Glu	Arg	Arg	Phe	Leu	Arg	Asn	Thr	Lys	Lys	Phe	Ile	Ser
			485						490					495	
Leu	Gly	Lys	His	Ala	Lys	Leu	Ser	Leu	Gln	Glu	Leu	Thr	Trp	Lys	Met
			500				505						510		
Ser	Val	Arg	Asp	Cys	Ala	Trp	Leu	Arg	Arg	Ser	Pro	Gly	Val	Gly	Cys
		515					520					525			
Val	Pro	Ala	Ala	Glu	His	Arg	Leu	Arg	Glu	Glu	Ile	Leu	Ala	Lys	Phe
	530					535					540				
Leu	His	Trp	Leu	Met	Ser	Val	Tyr	Val	Val	Glu	Leu	Leu	Arg	Ser	Phe
545				550						555					560
Phe	Tyr	Val	Thr	Glu	Thr	Thr	Phe	Gln	Lys	Asn	Arg	Leu	Phe	Phe	Tyr
			565						570					575	

Arg	Lys	Ser	Val	Trp	Ser	Lys	Leu	Gln	Ser	Ile	Gly	Ile	Arg	Gln	His
			580					585					590		
Leu	Lys	Arg	Val	Gln	Leu	Arg	Glu	Leu	Ser	Glu	Ala	Glu	Val	Arg	Gln
		595					600					605			
His	Arg	Glu	Ala	Arg	Pro	Ala	Leu	Leu	Thr	Ser	Arg	Leu	Arg	Phe	Ile
	610					615					620				
Pro	Lys	Pro	Asp	Gly	Leu	Arg	Pro	Ile	Val	Asn	Met	Asp	Tyr	Val	Val
625					630					635					640
Gly	Ala	Arg	Thr	Phe	Arg	Arg	Glu	Lys	Arg	Ala	Glu	Arg	Leu	Thr	Ser
				645					650					655	
Arg	Val	Lys	Ala	Leu	Phe	Ser	Val	Leu	Asn	Tyr	Glu	Arg	Ala	Arg	Arg
			660					665					670		
Pro	Gly	Leu	Leu	Gly	Ala	Ser	Val	Leu	Gly	Leu	Asp	Asp	Ile	His	Arg
		675					680					685			
Ala	Trp	Arg	Thr	Phe	Val	Leu	Arg	Val	Arg	Ala	Gln	Asp	Pro	Pro	Pro
	690					695					700				
Glu	Leu	Tyr	Phe	Val	Lys	Val	Asp	Val	Thr	Gly	Ala	Tyr	Asp	Thr	Ile
705					710					715					720
Pro	Gln	Asp	Arg	Leu	Thr	Glu	Val	Ile	Ala	Ser	Ile	Ile	Lys	Pro	Gln
				725					730					735	
Asn	Thr	Tyr	Cys	Val	Arg	Arg	Tyr	Ala	Val	Val	Gln	Lys	Ala	Ala	His
			740					745					750		
Gly	His	Val	Arg	Lys	Ala	Phe	Lys	Ser	His	Val	Ser	Thr	Leu	Thr	Asp
		755					760					765			
Leu	Gln	Pro	Tyr	Met	Arg	Gln	Phe	Val	Ala	His	Leu	Gln	Glu	Thr	Ser
						775					780				
Pro	Leu	Arg	Asp	Ala	Val	Val	Ile	Glu	Gln	Ser	Ser	Ser	Leu	Asn	Glu
785					790					795					800
Ala	Ser	Ser	Gly	Leu	Phe	Asp	Val	Phe	Leu	Arg	Phe	Met	Cys	His	His
				805				810						815	
Ala	Val	Arg	Ile	Arg	Gly	Lys	Ser	Tyr	Val	Gln	Cys	Gln	Gly	Ile	Pro
			820					825					830		
Gln	Gly	Ser	Ile	Leu	Ser	Thr	Leu	Leu	Cys	Ser	Leu	Cys	Tyr	Gly	Asp
		835					840					845			
Met	Glu	Asn	Lys	Leu	Phe	Ala	Gly	Ile	Arg	Arg	Asp	Gly	Leu	Leu	Leu
	850					855					860				
Arg	Leu	Val	Asp	Asp	Phe	Leu	Leu	Val	Thr	Pro	His	Leu	Thr	His	Ala
865					870					875					880
Lys	Thr	Phe	Leu	Arg	Thr	Leu	Val	Arg	Gly	Val	Pro	Glu	Tyr	Gly	Cys
				885					890					895	
Val	Val	Asn	Leu	Arg	Lys	Thr	Val	Val	Asn	Phe	Pro	Val	Glu	Asp	Glu

900					905					910					
Ala	Leu	Gly	Gly	Thr	Ala	Phe	Val	Gln	Met	Pro	Ala	His	Gly	Leu	Phe
	915						920					925			
Pro	Trp	Cys	Gly	Leu	Leu	Leu	Asp	Thr	Arg	Thr	Leu	Glu	Val	Gln	Ser
	930						935					940			
Asp	Tyr	Ser	Ser	Tyr	Ala	Arg	Thr	Ser	Ile	Arg	Ala	Ser	Leu	Thr	Phe
	945					950					955				960
Asn	Arg	Gly	Phe	Lys	Ala	Gly	Arg	Asn	Met	Arg	Arg	Lys	Leu	Phe	Gly
				965					970					975	
Val	Leu	Arg	Leu	Lys	Cys	His	Ser	Leu	Phe	Leu	Asp	Leu	Gln	Val	Asn
			980					985					990		
Ser	Leu	Gln	Thr	Val	Cys	Thr	Asn	Ile	Tyr	Lys	Ile	Leu	Leu	Leu	Gln
		995					1000					1005			
Ala	Tyr	Arg	Phe	His	Ala	Cys	Val	Leu	Gln	Leu	Pro	Phe	His	Gln	Gln
	1010					1015					1020				
Val	Trp	Lys	Asn	Pro	Thr	Phe	Phe	Leu	Arg	Val	Ile	Ser	Asp	Thr	Ala
	1025					1030					1035				1040
Ser	Leu	Cys	Tyr	Ser	Ile	Leu	Lys	Ala	Lys	Asn	Ala	Gly	Met	Ser	Leu
				1045					1050					1055	
Gly	Ala	Lys	Gly	Ala	Ala	Gly	Pro	Leu	Pro	Ser	Glu	Ala	Val	Gln	Trp
			1060					1065					1070		
Leu	Cys	His	Gln	Ala	Phe	Leu	Leu	Lys	Leu	Thr	Arg	His	Arg	Val	Thr
			1075					1080				1085			
Tyr	Val	Pro	Leu	Leu	Gly	Ser	Leu	Arg	Thr	Ala	Gln	Thr	Gln	Leu	Ser
	1090					1095					1100				
Arg	Lys	Leu	Pro	Gly	Thr	Thr	Leu	Thr	Ala	Leu	Glu	Ala	Ala	Ala	Asn
	1105					1110					1115				1120
Pro	Ala	Leu	Pro	Ser	Asp	Phe	Lys	Thr	Ile	Leu	Asp				
				1125					1130						

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1153 base pairs
 - (B) TYPE: Nucleotide
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTGCCTGCAG AGACCCGTCT GGTGCACTCT GATTCTCCAC TTGCCTGTTG CATGTCCTCG	60
TTCCCTTGTT TCTCACCACC TCTTGGGTTG CCATGTGCGT TTCCTGCCGA GTGTGTGTTG	120
ATCCTCTCGT TGCTCTCTGG TCACTGGGCA TTTGCTTTTA TTTCTCTTTG CTTAGTGTTA	180
CCCCCTGATC TTTTATTGT CGTTGTTTGC TTTTGTTTAT TGAGACAGTC TCACTCTGTC	240
ACCCAGGCTG GAGTGTAATG GCACAATCTC GGCTCACTGC AACCTCTGCC TCCTCGGTTT	300
AAGCAGTTCT CATTCCTCAA CCTCATGAGT AGCTGGGATT ACAGGCGCCC ACCACCACGC	360
CTGGCTAATT TTTGTATTTT TAGTAGAGAT AGGCTTTCAC CATGTTGGCC AGGCTGGTCT	420
CAAACCTCTG ACCTCAAGTG ATCTGCCCCG CTGGCCCTCC CACAGTGCTG GGATTACAGG	480
TGCAAGCCAC CGTGCCCGGC ATACCTTGAT CTTTAAAAAT GAAGTCTGAA ACATTGCTAC	540
CCTTGTCCTG AGCAATAAGA CCCTTAGTGT ATTTTAGCTC TGGCCACCCC CCAGCCTGTG	600
TGCTGTTTTT CCTGCTGACT TAGTTCTATC TCAGGCATCT TGACACCCCC ACAAGCTAAG	660
CATTATTAAT ATTGTTTTCC GTGTTGAGTG TTTCTTTAGC TTTGCCCCCG CCCTGCTTTT	720
CCTCCTTTGT TCCCCGTCTG TCTTCTGTCT CAGGCCCGCC GTCTGGGGTC CCCTTCCTTG	780
TCCTTTGCGT GGTTCCTCTG TCTTGTTATT GCTGGTAAAC CCCAGCTTTA CCTGTGCTGG	840
CCTCCATGGC ATCTAGCGAC GTCCGGGGAC CTCTGCTTAT GATGCACAGA TGAAGATGTG	900
GAGACTCACG AGGAGGGCGG TCATCTTGGC CCGTGAGTGT CTGGAGCACC ACGTGGCCAG	960
CGTTCCTTAG CCAGGGTTGG CTGTGTTCCG GCCGCAGAGC ACCGTCTGCG TGAGGAGATC	1020
CTGGCCAAGT TCCTGCACTG GCTGATGAGT GTGTACGTCG TCGAGCTGCT CAGGTCTTTC	1080
TTTATGTCA CGGAGACCAC GTTTCAAAAG AACAGGCTCT TTTCTACCG GAAGAGTGTC	1140
TGGAGCAAGT TGC	1153

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 412 base pairs
- (B) TYPE: Nucleotide
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CAGAGCCCTG GTCCTCCTGT CTCCATCGTC ACGTGGGCAC ACGTGGCTTT TCGCTCAGGA 60
 CGTCGAGTGG ACACGGTGAT CTCTGCCTCT GCTCTCCCTC CTGTCCAGTT TGCATAAACT 120
 TACGAGGTTC ACCTTCACGT TTTGATGGAC ACGCGGTTTC CAGGCACCGA GGCCAGAGCA 180
 GTGAACAGAG GAGGCTGGGC GCGGCAGTGG AGCCGGGTTG CCGGCAATGG GGAGAAGTGT 240
 CTGGAAGCAC AGACGCTCTG GCGAGGGTGC CTGCAGAGAC CCGCCTGGTG CACTCTGATT 300
 CTCCACTTGC CTGTTGCATG TCCTCGTTCC CTTGTTTCTC ACCACCTCTT GGGTTGCCAT 360
 GTGCGTTTCC TGCCGAGTGT GTGTTGATCC TCTCGTTGCC TCCTGGTCAC TG 412

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1012 base pairs
 (B) TYPE: Nucleotide
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGGGTCTTGG GCCACCCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT 60
 GTCACCTGCC AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG 120
 CCACTCCAC CCATCCGTGG GCCGCCAGCA CCACGCGGGC CCCCATCCA CATCGCGGCC 180
 ACCACGTCCC TGGGACACGC CTTGTCCCCC GGTGTACGCC GAGACCAAGC ACTTCTCTA 240
 CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA CTCAGCTCTC TGAGGCCAG 300
 CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCAGGC CCTGGATGCC 360
 AGGGACTCCC CGCAGGTTGC CCCGCTGCC CCAGCGCTAC TGGCAAATGC GGCCCTGTT 420
 TCTGGAGCTG CTTGGGAACC ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG 480
 CCCGCTGCGA GCTGCGGTCA CCCCAGCAGC CGGTGTCTGT GCCCAGGAGA AGCCCCAGGG 540
 CTCTGTGGCG GCCCCGAGG AGGAGGACAC AGACCCCGT CGCCTGGTGC AGCTGCTCCG 600
 CCAGCACAGC AGCCCTGGC AGGTGTACGG CTTCTGTCGG GCCTGCCTGC GCCGGCTGGT 660
 GCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC TTCCTCAGGA ACACCAAGAA 720
 GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT GGAAGATGAG 780
 CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 840

CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT 900
 CCTCCTGTCT CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC 960
 ACGGTGATCT CTGCCTCTGC TCTCCCTCCT GTCCAGTTTG CATAAACTTA CG 1012

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3972 base pairs
- (B) TYPE: Nucleotide
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAATTCGCGG CCGCGTCGAC GTTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC 60
 CCTGGCCCCG GCCACCCCG CGATGCCGCG CGCTCCCCGC TGCCGAGCCG TGCCTCCCT 120
 GCTGCGCAGC CACTACCGCG AGGTGCTGCC GCTGGCCACG TTCGTGCGGC GCCTGGGGCC 180
 CCAGGGCTGG CGGCTGGTGC AGCGCGGGGA CCCGGCGGCT TTCCGCGCGC TGGTGGCCCA 240
 GTGCCTGGTG TGCCTGCCCT GGGACGCACG GCCGCCCCC GCCGCCCTT CCTTCCGCCA 300
 GGTGTCCTGC CTGAAGGAGC TGGTGGCCCG AGTGCTGCAG AGGCTGTGCG AGCGCGGCGC 360
 GAAGAACGTG CTGGCCTTCG GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCCCCCCGA 420
 GGCTTCACC ACCAGCGTGC GCAGTACCT GCCCAACACG GTGACCGACG CACTGCGGGG 480
 GAGCGGGGCG TGGGGGCTGC TGCTGCGCCG CGTGGGCGAC GACGTGCTGG TTCACCTGCT 540
 GGCACGCTGC GCGCTCTTTG TGCTGGTGGC TCCCAGCTGC GCCTACCAGG TGTGCGGGCC 600
 GCCGCTGTAC CAGCTCGGCG CTGCCACTCA GGCCCGGCCC CCGCCACACG CTAGTGGACC 660
 CCGAAGGCGT CTGGGATGCG AACGGGCCTG GAACCATAGC GTCAGGGAGG CCGGGGTCCC 720
 CCTGGGCCTG CCAGCCCCGG GTGCGAGGAG GCGCGGGGGC AGTGCCAGCC GAAGTCTGCC 780
 GTTGCCCAAG AGGCCAGGC GTGGCGCTGC CCCTGAGCCG GAGCGGACGC CCGTTGGGCA 840
 GGGGTCCTGG GCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT 900
 GTCACCTGCC AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG 960
 CCACTCCCAC CCATCCGTGG GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC 1020

ACCACGTCCC	TGGGACACGC	CTTGTCCTCC	GGTGTACGCC	GAGACCAAGC	ACTTCCTCTA	1080
CTCCTCAGGC	GACAAGGAGC	AGCTGCGGCC	CTCCTTCCTA	CTCAGCTCTC	TGAGGCCAG	1140
CCTGACTGGC	GCTCGGAGGC	TCGTGGAGAC	CATCTTTCTG	GGTTCAGGC	CCTGGATGCC	1200
AGGGACTCCC	CGCAGGTTGC	CCCGCCTGCC	CCAGCGCTAC	TGGCAAATGC	GGCCCCTGTT	1260
TCTGGAGCTG	CTTGGGAACC	ACGCGCAGTG	CCCCTACGGG	GTGCTCCTCA	AGACGCACTG	1320
CCCCTGCGA	GCTGCGGTCA	CCCCAGCAGC	CGGTGTCTGT	GCCCGGGAGA	AGCCCCAGGG	1380
CTCTGTGGCG	GGCCCCGAGG	AGGAGGACAC	AGACCCCCGT	CGCCTGGTGC	AGCTGCTCCG	1440
CCAGCACAGC	AGCCCCCTGGC	AGGTGTACGG	CTTCGTGCGG	GCCTGCCTGC	GCCGGCTGGT	1500
GGCCCCAGGC	CTCTGGGGCT	CCAGGCACAA	CGAACGCCGC	TTCTCAGGA	ACACCAAGAA	1560
GTTTCATCTCC	CTGGGGAAGC	ATGCCAAGCT	CTCGCTGCAG	GAGCTGACGT	GGAAGATGAG	1620
CGTGCGGGAC	TGCGCTTGGC	TGCGCAGGAG	CCCAGGTGAG	GAGGTGGTGG	CCGTCGAGGG	1680
CCCAGGCCCC	AGAGCTGAAT	GCAGTAGGGG	CTCAGAAAAG	GGGGCAGGCA	GAGCCCTGGT	1740
CCTCCTGTCT	CCATCGTCAC	GTGGGCACAC	GTGGCTTTTC	GCTCAGGACG	TCGAGTGGAC	1800
ACGGTGATCT	CTGCCTCTGC	TCTCCCTCCT	GTCCAGTTTG	CATAAACTTA	CGAGGTTTAC	1860
CTTCACGTTT	TGATGGACAC	GCGGTTTCCA	GGCGCCGAGG	CCAGAGCAGT	GAACAGAGGA	1920
GGCTGGGCGC	GGCAGTGGAG	CCGGGTTGCC	GGCAATGGGG	AGAAGTGTCT	GGAAGCACAG	1980
ACGCTCTGGC	GAGGGTGCCT	GCAGGGGTTG	GCTGTGTTC	GGCCGCAGAG	CACCGTCTGC	2040
GTGAGGAGAT	CCTGGCCAAG	TTCCTGCACT	GGCTGATGAG	TGTGTACGTC	GTCGAGCTGC	2100
TCAGGTCTTT	CTTTTATGTC	ACGGAGACCA	CGTTTCAAAA	GAACAGGCTC	TTTTTCTACC	2160
GGAAGAGTGT	CTGGAGCAAG	TTGCAAAGCA	TTGGAATCAG	ACAGCACTTG	AAGAGGGTGC	2220
AGCTGCGGGA	GCTGTGCGAA	GCAGAGGTCA	GGCAGCATCG	GGAAGCCAGG	CCCGCCCTGC	2280
TGACGTCCAG	ACTCCGCTTC	ATCCCCAAGC	CTGACGGGCT	GCGGCCGATT	GTGAACATGG	2340
ACTACGTCGT	GGGAGCCAGA	ACGTTCCGCA	GAGAAAAGAG	GGTGGCTGTG	CTTTGGTTTA	2400
ACTTCCTTTT	TAAACAGAAG	TGCGTTTGAG	CCCCACATTT	GGTATCAGCT	TAGATGAAGG	2460
GCCCGGAGGA	GGGGCCACGG	GACACAGCCA	GGGCCATGGC	ACGGCGCCAA	CCCATTTGTG	2520
CGCACGGTGA	GGTGGCCGAG	GTGCCGGTGC	CTCCAGAAAA	GCAGCGTGGG	GGTGTAGGGG	2580
GAGCTCCTGG	GGCAGGGACA	GGCTCTGAGG	ACCACAAGAA	GCAGCTGGGC	CAGGGCCTGG	2640
ATGCAGCACG	GCCCGAGCGG	GTGGGGGCCC	ACCACGCCAT	TCTGGTCAAA	GGTGTGTAG	2700
TCGTAATAGC	CGGCCCAGGC	GCTCTGAACC	TTCAGAGTCT	CAAAAGCTGG	GACCCTCAGG	2760
GCCAAATGGG	GCCACACCTT	GTCCTGGAAG	AAATCATGGT	CCACTTCCAG	GTTCCCGGG	2820
TCCGGTTCTT	CCTGCTCAGT	GGGGCTACGA	CCACCTAGGT	AGTTGCTACC	TAATCCTTCC	2880

CGGCGAAAAT AGGCTCCACT GGTGTCTGCA ACAAGCGGAG TCTCTAGGCC TGGTCCCTGG 2940
 GGGCAGTGCC ACACATACAC ATACCTTTTC CTCGGCTCCA CAGGTAGCTT GGTGCCCTGC 3000
 AGGGTGCCAG GCGGCCCTC TCCAACACCA GCCAGTGCTG CGATTGCGC AGACCAGGCT 3060
 CCGGCTGCGT TGATCACAAT GGCGCATTCC ACAGGCTGGT ACTCCAGGCT GCGGTCCATC 3120
 TTCACATGGA CTTTCATGGAT CCTTTTCAAG ACCACCGCTT TGTCATCTGT GGTCAACATG 3180
 CGTTGAGATG AAGAGACAAA ACGTGTCAAC TCTCCCTGGC AGAAAAGGAC TCCCAAGGAC 3240
 TGGACCTTTC GCCGAAGCCC CTGGAGCAGA CACCAGGGGT CAAACCAACC TTCGTCCTCC 3300
 ATCCCATAG AGGCCAAAGC CACTCCCTCT GTGTTTATCC AGGGAAACTT GTTCCGAAGC 3360
 TGATCAGGAG ACATCAGAGA AACTTTGGCT CCCTCCTGCC TCTGCACTTT CACGTTGCTC 3420
 TCCATGGCTG CAGCATCCTT TTCTGAAGCC AGCAAGAGGT AGCCCGAGGG GTTGAACCGG 3480
 AGGTCCAGGG GAGGAGCATC GACTACGGCC AGGTACTCAT TGATGTTCCG TAGAAAGCTG 3540
 GCTGAAAAGA GGGAGAGCTG GATGTTCTCA GGCAATGAGA ACTGCTGACA AATCCCACCT 3600
 ACTGAGAGCC CAGTGGAGGC CTGTGAATAC GTGTGGTCCC GTTCCACCAC TAGCACTCGA 3660
 ATAGCACCTC GTCTGCTCTC CAGCTTCTTC AGCCAATAGG CCACAGACAA GCCAAGCACC 3720
 CCACCTCCCA CGATCACCAC ATCCGAGTGC TCGGGAGGCA GGTGGCTGGT GTCTTGCACT 3780
 AGATCACAGG ACCTTCCAGG CAGGATCGAC TTGATCTTCT TCTTAATCTC AGACACCTTT 3840
 CCATCCCAGT CCAGAGAAAA GCCTCCTCTG CGCGTGCCTG GCCTCCGGGT CAAGAGGCCC 3900
 CGGCCCATGC CGTGCGGCAG AACCTCCGA ATCATAGCCC CTCTGAGCCC GGGTCGACGC 3960
 GGCCGCGAAT TC 3972

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2089 base pairs
 - (B) TYPE: Nucleotide
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: cDNA
- (v) FRAGMENT TYPE: Linear
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCGGAAGAGT GTCTGGAGCA AGTTGCAAAG CATTGGAATC AGACAGCACT TGAAGAGGGT 60
 GCAGCTGCGG GAGCTGTCGG AAGCAGAGGT CAGGCAGCAT CGGGAAGCCA GGCCCGCCCT 120
 GCTGACGTCC AGACTCCGCT TCATCCCCAA GCCTGACGGG CTGCGGCCGA TTGTGAACAT 180

GGACTACGTC	GTGGGAGCCA	GAACGTTCCG	CAGAGAAAAG	AGGGCCGAGC	GTCTCACCTC	240
GAGGGTGAAG	GCACTGTTCA	GCGTGCTCAA	CTACGAGCGG	GCGCGGCGCC	CCGGCCTCCT	300
GGGCGCCTCT	GTGCTGGGCC	TGGACGATAT	CCACAGGGCC	TGGCGCACCT	TCGTGCTGCG	360
TGTGCGGGCC	CAGGACCCGC	CGCCTGAGCT	GTACTTTGTC	AAGGTGGATG	TGACGGGCGC	420
GTACGACACC	ATCCCCCAGG	ACAGGCTCAC	GGAGGTCATC	GCCAGCATCA	TCAAACCCCA	480
GAACACGTAC	TGCGTGCGTC	GGTATGCCGT	GGTCCAGAAG	GCCGCCCATG	GGCACGTCCG	540
CAAGGCCTTC	AAGAGCCACG	TCTCTACCTT	GACAGACCTC	CAGCCGTACA	TGCGACAGTT	600
CGTGCTCAC	CTGCAGGAGA	CCAGCCCGCT	GAGGGGTGCC	GTCGTCATCG	AGCAGAGCTC	660
CTCCCTGAAT	GAGGCCAGCA	GTGGCCTCTT	CGACGTCTTC	CTACGCTTCA	TGTGCCACCA	720
CGCCGTGCGC	ATCAGGGGCA	AGTCCTACGT	CCAGTGCCAG	GGGATCCCGC	AGGGCTCCAT	780
CCTCTCCACG	CTGCTCTGCA	GCCTGTGCTA	CGGCGACATG	GAGAACAAGC	TGTTTGCGGG	840
GATTCGGCGG	GACGGGCTGC	TCCTGCGTTT	GGTGGATGAT	TTCTTGTTGG	TGACACCTCA	900
CCTCACCCAC	GCGAAAACCT	TCCTCAGGAC	CCTGGTCCGA	GGTGTCCCTG	AGTATGGCTG	960
CGTGGTGAAC	TTGCGGAAGA	CAGTGGTGAA	CTTCCCTGTA	GAAGACGAGG	CCCTGGGTGG	1020
CACGGCTTTT	GTTCAGATGC	CGGCCACGG	CCTATTCCCC	TGGTGCGGCC	TGCTGCTGGA	1080
TACCCGGACC	CTGGAGGTGC	AGAGCGACTA	CTCCAGCTAT	GCCCGGACCT	CCATCAGAGC	1140
CAGTCTCACC	TTCAACCGCG	GCTTCAAGGC	TGGGAGGAAC	ATGCGTCGCA	AACTCTTTGG	1200
GGTCTTGCGG	CTGAAGTGTC	ACAGCCTGTT	TCTGGATTTG	CAGGTGAACA	GCCTCCAGAC	1260
GGTGTGCACC	AACATCTACA	AGATCCTCCT	GCTGCAGGCG	TACAGGTTTC	ACGCATGCGT	1320
GCTGCAGCTC	CCATTTTCATC	AGCAAGTTTG	GAAGAACCCC	ACATTTTTC	TGCGCGTCAT	1380
CTCTGACACG	GCCTCCCTCT	GCTACTCCAT	CCTGAAAGCC	AAGAACGCAG	GTATGTGCAG	1440
GTGCCTGGCC	TCAGTGGCAG	CAGTGCCTGC	CTGCTGGTGT	TAGTGTGTCA	GGAGACTGAG	1500
TGAATCTGGG	CTTAGGAAGT	TCTTACCCCT	TTTCGCATCA	GGAAGTGGTT	TAACCCAACC	1560
ACTGTCAGGC	TCGTCTGCCC	GCCCTCTCGT	GGGGTGAGCA	GAGCACCTGA	TGGAAGGGAC	1620
AGGAGCTGTC	TGGGAGCTGC	CATCCTTCCC	ACCTTGCTCT	GCCTGGGGAA	GCGCTGGGGG	1680
GCCTGGTCTC	TCCTGTTTGC	CCCATGGTGG	GATTTGGGGG	GCCTGGCCTC	TCCTGTTTGC	1740
CCTGTGGTGG	GATTGGGCTG	TCTCCCGTCC	ATGGCACTTA	GGGCCCTTGT	GCAAACCCAG	1800
GCCAAGGGCT	TAGGAGGAGG	CCAGGCCCAG	GCTACCCAC	CCCTCTCAGG	AGCAGAGGCC	1860
GCGTATCACC	ACGACAGAGC	CCGCGCCCGT	CCTCTGCTTC	CCAGTCACCG	TCCTCTGCCC	1920
CTGGACACTT	TGTCCAGCAT	CAGGGAGGTT	TCTGATCCGT	CTGAAATTCA	AGCCATGTCG	1980
AACCTGCGGT	CCTGAGCTTA	ACAGCTTCTA	CTTTCTGTTC	TTTCTGTGTT	GTGGAGACCC	2040

TGAGAAGGAC CCTGGGAGCT CTGGGAATTT GGAGTGACCA AAGGTGTGC

2089

Patent Claims

1. Catalytically active human telomerase subunit, its functional equivalents, its variants and its catalytically active fragments.
2. Telomerase according to Claim 1, comprising the amino acid sequence depicted in Fig. 1b or its functional equivalents.
3. Nucleic acid sequences encoding compounds according to Claims 1 and 2 and their functional equivalents.
4. Nucleic acid sequences according to Claim 3, comprising the DNA sequence depicted in Fig. 1a or its functional equivalents.
5. Antisense nucleic acids binding to the nucleic acid sequence according to Claim 3 or 4.
6. Antibodies against telomerase according to Claims 1 and 2, where appropriate labelled with one or more labels.
7. Use of nucleic acid sequences according to Claims 3 and 4 for preparing telomerase.
8. Use of antibodies according to Claim 6 for diagnosis.
9. Use of antibodies according to Claim 6 for preparing medicaments.
10. Vector comprising a nucleic acid sequence, in particular DNA, according to Claims 3 and 4.
11. Microorganisms harbouring the vector according to Claim 10.

- 5

Catalytic subunit of human telomerase and its diagnostic and therapeutic use

Abstract

5

This invention relates to the nucleotide sequence, and the protein sequence which is deduced from it, which encodes the catalytic subunit of human telomerase. In addition, this invention relates to methods which involve a pharmaceutical, diagnostic or therapeutic use of this

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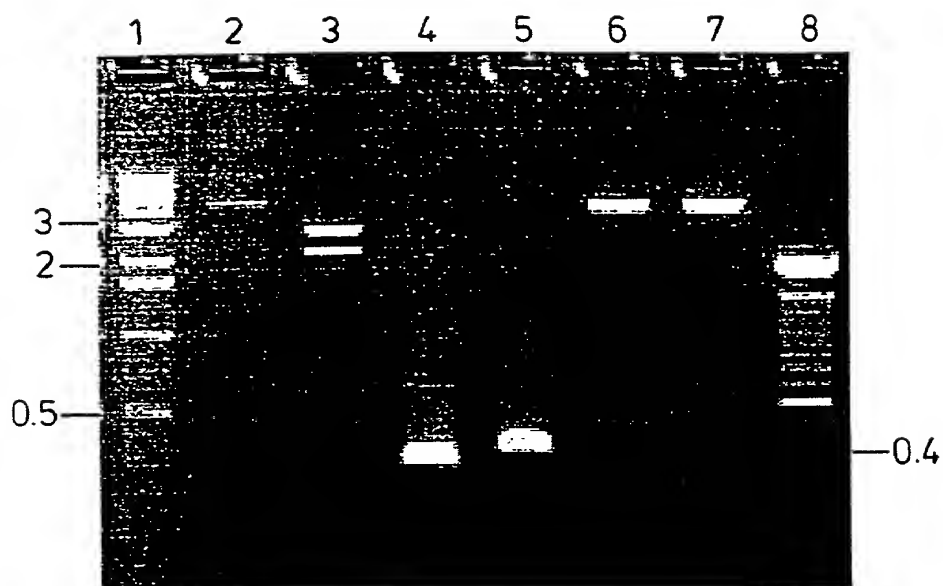
gene/protein, especially in the treatment of cancer and ageing.

Fig. 1

GTTCACGGCA	GCCTGCGCTC	CTGCTGCGCA	CGTGGGAAGC	CTGGCCCCCG	GCCACCCCCG	CGATGCCCGC	70
GCTTCCCCGC	TGCCGAGCCG	TGCGTCCCT	GCTGCGCAGC	CACTACCGCG	AGGTGCTGCC	GCTGGCCACG	140
TTCGTGCGGC	GCCTGGGGCC	CCAGGGCTGG	CGGTGGTGC	AGCGGGGGA	CCCGGCGGCT	TTCCGCGCGC	210
TGGTGGCCCA	GTGCCTGGTG	TGCGTGCCCT	GGGACGCAGC	GCCGCCCCCC	GCCGCCCCCT	CCTTCCGCCA	280
GGTGTCTTGC	CTGAAGGAGC	TGGTGGCCCG	AGTGCTGCAG	AGGTGTGCG	AGCGCGGCGC	GAAGAACGTG	350
CTGGCCTTCG	GCTTCGCGCT	GTGGACGGG	GCCC CGGGG	GCCCCCCGA	GGCCTTACC	ACCAGCGTGC	420
GCAGCTACCT	GCCCAACACG	GTGACCGACG	CACTGCGGGG	GAGCGGGGCG	TGGGGGCTGC	TGCTGCGCCG	490
CGTGGGCGAC	GACGTGCTGG	TTCACTTGCT	GGCAGCTGTC	GCCTCTTTG	TGCTGGTGGC	TCCCAGCTGC	560
GCCTACCAGG	TGTGCGGGCC	GCCGCTGTAC	CAGCTCGGCG	CTGCCACTCA	GGCCCCGGCC	CCGCCACACG	630
CTAGTGGAAC	CCGAAGGCGT	CTGGGATGCG	AACGGGCTG	GAACTACTAG	GTACGGGAGC	CCGGGCTCCC	700
CCTGGGCGCTG	CCAGCCCCGG	GTGCGAGGAG	GCGCGGGGCG	AGTGCCAGCC	GAAGTCTGCC	GTTGCCCAAG	770
AGGCCAGGC	GTGGCGCTGC	CCCTGAGCCG	GAGCGGACGC	CCGTTGGGCA	GGGGTCTTGG	GCCACCCCGG	840
GCAGGACGCG	TGGACCGAGT	GACCGTGGTT	TCTGTGTGGT	GTCACTTGCC	AGACCCGCGC	AAGAAGCCAC	910
CTCTTTGGAG	GGTGCCTCT	CTGGCACGCG	CCACTCCCAC	CCATCGTGG	GCGGCCAGCA	CCACGCGGGC	980
CCCCCATCCA	CATCGCGGCC	ACCAGTCCC	TGGGACACGC	CTTGTCCTCC	GGTGTACGCC	GAGACCAAGC	1050
ACTTCTCTA	CTCTCAGGC	GACAAGGAGC	AGCTGCGGCC	CTCTTCTCTA	CTCAGCTCTC	TGAGGCCCCAG	1120
CCTGACTGGC	GCTCGGAGGC	TCGTGGAGAC	CATCTTTCTG	GGTTCAGGC	CCTGGATGCC	AGGGACTCCC	1190
CGCAGGTTGC	CCCCGCTGCC	CCAGCGCTAC	TGGCAAATGC	GGCCCTGTT	TCTGGAGCTG	CTTGGGAACC	1260
ACGCGCAGTG	CCCTACGGG	GTGCTCCTCA	AGACGCACTG	CCCGCTGCGA	GCTGCGGTCA	CCCCAGCAGC	1330
CGGTGTCTGT	GCCCCGGAGA	AGCCCCAGGG	CTCTGTGGCG	GCCCCCGAGG	AGGAGGACAC	AGACCCCCGT	1400
CGCTGGTGC	AGCTGCTCCG	CCAGCACAGC	AGCCCTGCG	AGGTGTACGG	CTTCGTGCGG	GCCTGCCCTG	1470
GCCGGCTGGT	GCCCCCAGGC	CTCTGGGCT	CACGGGACCA	GGAAGCGCCG	TTCTCAGGA	ACACCAAGAA	1540
GTTTCATCTCC	TGCGGGAAGC	ATGCGAAGCT	CTCGCTCAG	CGAGTGACGT	GGAAGATGAG	CGTGCGGGAC	1610
TGCGCTTGGC	CTGCGAGGAG	CCAGGGGTT	GGCTGTGTTT	CGGCCGCGA	GCACCGTCTG	CGTGAGGAGA	1680
TCTTGCCAA	GTTCTGAC	TGGCTGATGA	GTGTGTACGT	CGTCGAGCTG	CTCAGGTCTT	TCTTTTATGT	1750
CACGGAGACC	ACGTTTCAA	AGAACAGGCT	CTTTTCTAC	CGGAAGAGTG	TCTGGAGCAA	GTTGCAAAGC	1820
ATTGGAATCA	GACAGCACTT	GAAGAGGGTG	CAGCTGCGGG	AGCTGTCCGA	AGCAGAGGTC	AGGCAGCATC	1890
GGGAAGCCAG	GCCCGCCCTG	CTGACGTCCA	GACTCCGCTT	CATCCCCAAG	CCTGACGGGC	TGCGGCCGAT	1960
TGTGAACATG	GACTACGTGC	TGGGAGCCAG	AACGTTCCGC	AGAGAAAAGA	GGGCCGAGCG	TCTCACCTCG	2030
AGGGTGAAGG	CACTGTTTCA	CGTGCTCAAC	TACGAGCGGG	CGCGCGCCCC	CGGCCTCTG	GGCGCCTCTG	2100
TGCTGGGCCT	GGACGATATC	CACAGGGCCT	GGCGCACCTT	CGTGCTGCGT	GTGCGGGCCC	AGGACC CGCC	2170
GCCTGAGCTG	TACTTTGTCA	AGGTGGATGT	GACGGGCGCG	TACGACACCA	TCCCCCAGGA	CAGGCTCACG	2240
GAGGTCATCG	CCAGCATCAT	CAAACCCAG	AACAGCTACT	CGTGCGTCTG	GTATGCCCTG	GTCCAGAAGG	2310
CCGCCCATCG	GCACGTCCGC	AAGCGCTTCA	AGAGCCACGT	CTCTACTCTG	ACAGACCTCC	AGCCGTACAT	2380
GCCACAGTTT	GTGGCTCAC	TAGCGGAGAC	CAGCCCGCTG	AGGGATGCCG	TCGTCTATCGA	GCAGAGCTCC	2450
TCCCTGAATG	AGGCCAGCAG	TGGCCTCTTC	GACGTCTTCC	TACGCTTCAT	GTGCCACCAC	GCCGTGCGCA	2520
TCAGGGGCAA	GTCTTACGTC	CAGTGCCAGG	GGATCCCGCA	GGGCTCCATC	CTCTCCACGC	TGCTCTGCAG	2590
CCTGTGCTAC	GGCGACATGG	AGAAACAGCT	GTTTGCGGGG	ATTGCGGGG	ACGGGCTGCT	CCTGCGTTTG	2660
GTGGATGATT	TCTTGTGGT	GACACCTCAC	CTCACCCACG	CGAAAACCTT	CCTCAGGACC	CTGGTCCGAG	2730
GTGTCCCTGA	GTATGGCTGC	GTGGTGAAC	TGCGGAAGAC	AGTGGTGAAC	TTCCCTGTAG	AAGACGAGGC	2800
CCTGGGTGGC	ACGGCTTTTG	TTCAGATGCC	GGCCACCGG	CTATTTCCCT	GGTGCGGCTG	GCTGCTGGAT	2870
ACCCGACCC	TGGAGGTGCA	GAGCGACTAC	TCCAGTATG	CCCGGACCTC	CATCAGAGCC	AGTCTCACCT	2940
TCAACCGCG	CTTCAAGGCT	GGGAGGAACA	TGCGTCGCAA	ACTCTTTGGG	GTCTTGCGGC	TGAAGTGTCA	3010
CAGCCTGTTT	CTGGATTTCG	AGGTGAACAG	CCTCCAGACG	GTGTGCACCA	ACATCTACAA	GATCCTCCTG	3080
CTGACCGGCT	ACAGGTTTCA	CGCATGTGTG	CTGCAGCTCC	CATTTCTATCA	GCAAGTTTGG	AAGAACCCCA	3150
CATTTTCTCT	GCGCGTCTATC	TCTGACACGG	CCTCCCTCTG	CTACTCCATC	CTGAAAGCCA	AGAACGCAGG	3220
GATGTGCTG	GGGGCCAAAG	GCGCGCGCGG	CCCTCTGCCC	TCCGAGGCCG	TGCAGTGGCT	GTGCCACCAA	3290
GCATTCTCTG	TCAAGCTGAC	TCGACACCGT	GTCACTTACG	TGCCACTCCT	GGGGTCACTC	AGGACAGCCC	3360
AGACGCAGCT	GAGTCGGAAG	CTCCCGGGGA	CGACGTGAC	TGCCCTGGAG	GCCGCAGCCA	ACCCGGCACT	3430
GCCCTCAGAC	TTCAAGACCA	TCCTGGACTG	ATGGCCACCC	GCCACAGCC	AGGCCGAGAG	CAGACACCAG	3500
CAGCCCTGTC	ACGCCCGGCT	CTACGTCCCA	GGGAGGGAGG	GGCGGCCAC	ACCCAGGCC	GCACCGCTGG	3570
GAGTCTGAGG	CCTGAGTGAG	TGTTTGCCG	AGGCCTGCAT	GTCCGGCTGA	AGGCTGAGTG	TCCGGCTGAG	3640
GCCTGAGCGA	GTGTCCAGCC	AAGGGCTGAG	TGTCCAGCAG	ACCTCGCGCT	TTCACTTCCC	CACAGGCTGG	3710
CGCTCGGCTC	CACCCACGGG	CCAGCTTTTC	CTCACCAAGG	GCCCGGCTTC	CACTCCCCAC	ATAGGAATAG	3780
TCCATCCCCA	GATTTCGCAT	TGTTTACCCC	TCGCCCTGCC	CTCCTTTGCC	TTCCACCCCC	ACCATCCAGG	3850
TGGAGACCC	GAGAAGGACC	CTGGGAGCTC	TGGGAATTTG	GAGTGACCAA	AGGTGTGCCC	TGTACACAGG	3920
CGAGGACCC	GCACCTGGAT	GGGGGTCCCT	GTGGGTCAA	TTGGGGGGAG	GTGCTGTGGG	AGTAAATAC	3990
TGAATATATG	AGTTTTTCAG	TTTTGAAAAA	AAAAAAAAAA	AAAAAAAAAA	AA		4042

Fig. 2

MPRAPRCRAV	RSLLRSHYRE	VLPLATFVRR	LGPQGWRLVQ	RGDPAAFRAL	50
VAQCLVCVPW	DARPPPAAPS	FRQVSCLKEL	VARVLQRLCE	RGAKNVLAFG	100
FALLDGARGG	PPEAFTTSVR	SYLPNTVTDA	LRGSGAWGLL	LRRVGDDVLV	150
HLLARCALFV	LVAPSCAYQV	CGPLYQLGA	ATQARPPPHA	SGPRRRLGCE	200
RAWNHSVREA	GVPLGLPAPG	ARRRGGSASR	SLPLPKRPRR	GAAPEPERTP	250
VGQGSWAHPG	RTRGPSDRGF	CVVSPARPAE	EATSLEGALS	GTRHSHPSVG	300
RQHHAGPPST	SRPPRPWDTP	CPPVYAETKH	FLYSSGDKEQ	LRPSFLLSSL	350
RPSLTGARRL	VETIFLGSRP	WMPGTPRRLP	RLPQRYWQMR	PLFLELLGNH	400
AQCPYGVLLK	THCPLRAAVT	PAAGVCAREK	PQGSVAAPEE	EDTDPRRLVQ	450
LLRQHSSPWQ	VYGFVRACLR	RLVPPGLWGS	RHNERRFLRN	TKKFISLGKH	500
AKLSLQELTW	KMSVRDCAWL	RRSPGVGCVP	AAEHRLREEI	LAKFLHWLMS	550
VYVVELLRSF	FYVTETTFQK	NRLFFYRKSV	WSKLQSIGIR	OHLKRVQLRE	600
LSEAEVRQHR	EARPALLTSR	LRFIPKPDGL	RPIVNMDYVV	GARTFRREKR	650
AERLTSRVKA	LFSVLNYERA	RRPGLLGASV	LGLDDIHRAW	RTFVLRVRAQ	700
DPPPELYFVK	VDVTGAYDTI	PQDRLTEVIA	SIKPQNTYC	VRRYAVVQKA	750
AHGHRKAFK	SHVSTLTDLQ	PYMRQFVAHL	QETSPLRDAV	VIEQSSSLNE	800
ASSGLFDVFL	RFMCHHAVRI	RGKSYVQCQG	IPQGSILSTL	LCSLCYGDME	850
NKLFAGIRRD	GLLLRLVDDF	LLVTPHLTHA	KTFLRTLVRG	VPEYGCVVNL	900
RKTVVNFPVE	DEALGGTAFV	QMPAHGLFPW	CGLLLDTRTL	EVQSDYSSYA	950
RTSIRASLTF	NRGFKAGRNM	RRKLFGLVRL	KCHSLFLDLQ	VNSLQTVCTN	1000
IYKILLQAY	RFHACVLQLP	FHQQVWKNPT	FFLRVISDTA	SLCYSILKAK	1050
NAGMSLGAKG	AAGPLPSEAV	QWLCHQAFLL	KLTRHRVTYV	PLLGSRLTAQ	1100
TQLSRKLPGT	TLTALEAAAN	PALPSDFKTI	LD		1132

Fig. 3

Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>129)

	Seq1(1>129)	Seq2(1>150)	Similarity	Gap	Gap Consensus
1	100	100	100	0	100
2	100	100	100	0	100
3	100	100	100	0	100
4	100	100	100	0	100
5	100	100	100	0	100
6	100	100	100	0	100
7	100	100	100	0	100
8	100	100	100	0	100
9	100	100	100	0	100
10	100	100	100	0	100
11	100	100	100	0	100
12	100	100	100	0	100
13	100	100	100	0	100
14	100	100	100	0	100
15	100	100	100	0	100
16	100	100	100	0	100
17	100	100	100	0	100
18	100	100	100	0	100
19	100	100	100	0	100
20	100	100	100	0	100
21	100	100	100	0	100
22	100	100	100	0	100
23	100	100	100	0	100
24	100	100	100	0	100
25	100	100	100	0	100
26	100	100	100	0	100
27	100	100	100	0	100
28	100	100	100	0	100
29	100	100	100	0	100
30	100	100	100	0	100
31	100	100	100	0	100
32	100	100	100	0	100
33	100	100	100	0	100
34	100	100	100	0	100
35	100	100	100	0	100
36	100	100	100	0	100
37	100	100	100	0	100
38	100	100	100	0	100
39	100	100	100	0	100
40	100	100	100	0	100
41	100	100	100	0	100
42	100	100	100	0	100
43	100	100	100	0	100
44	100	100	100	0	100
45	100	100	100	0	100
46	100	100	100	0	100
47	100	100	100	0	100
48	100	100	100	0	100
49	100	100	100	0	100
50	100	100	100	0	100
51	100	100	100	0	100
52	100	100	100	0	100
53	100	100	100	0	100
54	100	100	100	0	100
55	100	100	100	0	100
56	100	100	100	0	100
57	100	100	100	0	100
58	100	100	100	0	100
59	100	100	100	0	100
60	100	100	100	0	100
61	100	100	100	0	100
62	100	100	100	0	100
63	100	100	100	0	100
64	100	100	100	0	100
65	100	100	100	0	100
66	100	100	100	0	100
67	100	100	100	0	100
68	100	100	100	0	100
69	100	100	100	0	100
70	100				

P123.PRO	P123.PRO	Index	Number	Length
PHTC.PRO	PHTC.PRO	Index	Number	Length

(2>124)	(1>117)	31.5	4	6	123
---------	---------	------	---	---	-----

PHTC.PRO	KFLHWLMSVYVVVELLSFFYVTETTFQKNRLFFYRKSVWSKLSQSIGIRQHLKRVQLRDVSEAEVRQHRREARPALLTSRLR	↖10	↖20	↖30	↖40	↖50	↖60	↖70	↖80
	K:L:W:... VV:L:R:FFYVTE :: ::YRK::W: :: ::I ::LK: L :V E EV :: :: ::LR								
P123.PRO	KLLRWIFEDLVSLIRCFFYVTEQOKSYSKTYYYRKNIWDVIMKMSI -ADLKKETLAEVOEKEV -EEWKKSLGFAPGKLR	↖10	↖20	↖30	↖40	↖50	↖60	↖70	
PHTC.PRO	FIPKPDGLRPIVNM DYVVGARTFRREKRAERLTSRVKALFSVLNYERA	↖90	↖100	↖110	↖120				
	:IPK ::::RPI M.: . . . :::::LT:::K L S L :								
P123.PRO	LIPKKTIFRPI --MIF --NKKIVNSDRKTTKLTNTKLLNSHLMKLT	↖80	↖90	↖100	↖110	↖120			

Fig. 5

Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>150) Seq2(1>150) Similarity Gap Consensus

P123.PRO EST2P.PRO Index Number Length Length

(2>148)	(1>146)	21.6	4	5	149
P123.PRO	LLRWIFEDLVVSLIRCFYVTEQ0KSYSKYTYRKNIDVIMKMSIADLKKETLAEVQEEVEEKKSLGFAPGKLRIP	↖10	↖20	↖30	↖40
EST2P.PRO	FISWLFRLIPKIIQIFFCYCIEISSTVIYF-RHDTWNLITPFIVEYFKTYLVENNVCNRNHSYTLNHNHSMRIIP	↖10	↖20	↖30	↖40
P123.PRO	KKT--TFRPIMTFNKKIVNSDRKTTKLTINTKLLNSHMLKTLKNRMFKDPFGFAVFNYYDDVMKKYEEFVC	↖90	↖100	↖110	↖120
EST2P.PRO	KK: .FR I . : : : : K . : : : : L: N: : : : : .F: : : : : .EF	↖80	↖90	↖100	↖110

PHTC.PRO	EST2P.PRO	Index	Number	Length	
(3>85)	(1>80)	23.3	3	83	
PHTC . PRO	FLHWLMSVYVVELLRSFFYYVTETTFQKNRLFFYRKSWSKLOSIGIROHLKRVLDRDVS EAEVRQHREARPALITSRLRF F : WL.. : FFY TE.: . : : R...W:KL : I : : K L : : : .. : : : S : : R:				
EST2P . PRO	FISWLFRQLIPKI IOTFFCYCTEIS-STVTIVYFRHDTWNKLITPFIVEYFKTY-LVE-NNVCRNHNHSYTL SNFNHSKMRI				
PHTC . PRO	IPKPDGLRP IVNMDYVVGARTFRREKRAERLT SRVKALFSVLNYERA IPK .. : GA . . E . : : : : L: Y R.				
EST2P . PRO	IPKKSNNEFRI IAIPCRGADEEEFTIYKENHKNAIOPTOKILEYLRN				

Alignment Workspace of Untitled, using Clustal method with PAM250 residue weight table

	16	20	30	40	50	60	70	80
-KFLXWLFXXLVVLIRXFFYVTEXXXXXSXXXXXXXXYRKKXWXKLXXXXIXXLKXXXVEXEVRHXHXXLX-FXXS								
PHTC.PRO	AKFLHWLMSVVVELLRSEFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLMKVQLRDVSEAEVRQHRARPA-LLTS							
P123.PRO	-KLLRWIFEDLVSLIRCFFYVTEQQKSYSKTYRYRNKIWDVIMKMSI-ADLKKETLAEVQEKEV-EEWKKSIG-FAPG							
EST2P.PRO	--FISWLFRQLIPKIIQTFFCYCTEIS-STVTIVYFRHDTWNKLITPFIVEYFKTYL--VENNVCRNHNSYTLSNFNHS							
	96	100	110	120	130	140	150	160
LRXIPKGGXX--FRPLXXXXXXXXXX---								
PHTC.PRO	LRFIKPDPG--LRPIVNMDYVGAR---TFRREKRAERLITSRVKAL-----FSVLNYERA							
P123.PRO	LRLIPKKT--FRPIMTFN----KK----IVNSDRKTTKLTTNTKLLNSHLMLKTLKNRMFKDPPGFAGAVFNYYDDVMKKYE							
EST2P.PRO	MRIIPKKSNNEFR-IIAIPCRCGADEEEFTIYKENHNKAIQPTOKILE---YLNRKRPTSFTKI--YSPTQIADRIKEFK							

GTGCCTGCAG	AGACCCGTCT	GGTGCACTCT	GATTCTCCAC	TTGCCTGTTG	CATGTCCTCG	TTCCCTTGTT	70
TCTCACCACC	TCTTGGGTTG	CCATGTGCGT	TTCTGCCGA	GTGTGTGTTG	ATCCTCTCGT	TGCCCTCTGG	140
TCACTGGGCA	TTTGCTTTTA	TTTCTCTTTG	CTTAGTGTTA	CCCCCTGATC	TTTTTATTGT	CGTTGTTTGC	210
TTTTGTTTAT	TGAGACAGTC	TCACTCTGTC	ACCCAGGCTG	GAGTGTAATG	GCACAATCTC	GGCTCACTGC	280
AACCTCTGCC	TCCTCGGTTT	AAGCAGTTCT	CATTCTCTAA	CCTCATGAGT	AGCTGGGATT	ACAGGCGCCC	350
ACCACCACGC	CTGGCTAATT	TTTGTATTTT	TAGTAGAGAT	AGGCTTTTCA	CATGTTGGCC	AGGCTGGTCT	420
CAAACCTCTG	ACCTCAAGTG	ATCTGCCCCG	CTTGGCCTCC	CACAGTGCTG	GGATTACAGG	TGCAAGCCAC	490
CGTGCCCGGC	ATACCTTGAT	CTTTTAAAAAT	GAAGTCTGAA	ACATTGCTAC	CCTTGTCTTG	AGCAATAAGA	560
CCCTTAGTGT	ATTTTAGCTC	TGGCCACCCC	CCAGCCTGTG	TGCTGTTTTT	CCTGCTGACT	TAGTTCCTATC	630
TCAGGCATCT	TGACACCCCC	ACAAGCTAAG	CATTATTAAT	ATTGTTTTTCC	GTGTTGAGTG	TTTCTTTAGC	700
TTTGCCCCCG	CCCTGCTTTT	CCTCCTTTGT	TCCCCGCTG	TCTTCTGTCT	CAGGCCCCGC	GTCTGGGGTC	770
CCCTTCCTTG	TCCTTTGCGT	GGTTCTTCTG	TCTTGTTATT	GCTGGTAAAC	CCCAGCTTTA	CCTGTGCTGG	840
CCTCCATGGC	ATCTAGCGAC	GTCCGGGGAC	CTCTGCTTAT	GATGCACAGA	TGAAGATGTG	GAGACTCAGG	910
AGGAGGGCGG	TCATCTTGGC	CCGTGAGTGT	CTGGAGCACC	ACGTGGCCAG	CGTTCCTTAG	CCAGGGTTGG	980
CTGTGTTCCG	GCCGCAGAGC	ACCGTCTGCG	TGAGGAGATC	CTGGCCAAAGT	TCCTGCACTG	GCTGATGAGT	1050
GTGTACGTCG	TCGAGCTGCT	CAGGTCTTTC	TTTTATGTCA	CGGAGACCAC	GTTTCAAAAG	AACAGGCTCT	1120
TTTTCTACCG	GAAGAGTGTC	TGGAGCAAGT	TGC				1153

Fig. 9

CAGAGCCCTG	GTCCTCCTGT	CTCCATCGTC	ACGTGGGCAC	ACGTGGCTTT	TGCTCAGGA	CGTCGAGTGG	70
ACACGGTGAT	CTCTGCCCTCT	GCTCTCCCTC	CTGTCCAGTT	TGCATAAACT	TACGAGGTTT	ACCTTCACGT	140
TTTGATGGAC	ACGCGGTTTC	CAGGCACCGA	GGCCAGAGCA	GTGAACAGAG	GAGGCTGGGC	CGGGCAGTGG	210
AGCCGGGTTG	CCGGCAATGG	GGAGAAGTGT	CTGGAAGCAC	AGACGCTCTG	GCGAGGGTGC	CTGCAGAGAC	280
CCGCTCGGTG	CACCTCGATT	CTCCACTTGC	CTGTTGCATG	TCCTCGTTCC	CTTGTTTCTC	ACCACCTCTT	350
GGGTTGCCAT	GTGCGTTTTCC	TGCCGAGTGT	GTGTTGATCC	TCTCGTTGCC	TCCTGGTCC	TG	412

Fig. 10

GGGGTCCCTGG	GCCCACCCCG	GCAGGACCGG	TGGACCGAGT	GACCGTGGTT	TCTGTGTGGT	GTCACCTGCC	70
AGACCCGCGG	AAGAAGCCAC	CTCTTTGGAG	GGTGCCTCT	CTGGCACGGG	CCACTCCCAC	CCATCCGTGG	140
GCCGCCAGCA	CCACGCGGGC	CCCCATCCA	CATCGCGGCC	ACCACGTCCC	TGGGACACGC	CTTGTCCCCC	210
GGTGACGCC	GAGACCAAGC	ACTTCCTCTA	CTCCTCAGGC	GACAAGGAGC	AGCTGCGGcC	CTCCTTCCTA	280
CTCAGCTCTC	TGAGGCCCAG	CCTGACTGGC	GCTCGGAGGC	TGCTGGAGAC	CATCTTTCTG	GGTTCCAGGC	350
CCTGGATGCC	AGGGACTCCC	CGCAGGTTGC	CCCGCCTGCC	CCAGCGCTAC	TGGCAAATGC	GGCCCCCTGTT	420
TCTGGAGCTG	CTTGGGAACC	ACGCGCAGTG	CCCCTACGGG	GTGCTCCTCA	AGACGCACTG	CCCCGTGCGA	490
GCTGCGGTCA	CCCCAGCAGC	CGGTGTCTGT	GCCCCGGAGA	AGCCCCAGGG	CTCTGTGGCG	GCCCCCGAGG	560
AGGAGGACAC	AGACCCCGT	CGCCTGGTGC	AGCTGCTCCG	CCAGCACAGC	AGCCCCCTGGC	AGGTGTACGG	630
CTTCGTGCGG	GCCTGCCTGC	GCCGGCTGGT	GCCCCCAGGC	CTCTGGGGCT	CCAGGCACAA	CGAACGCCGC	700
TTCTCTAGGA	ACACCAAGAA	GTTCATCTCC	CTGGGGAAGC	ATGCCAAGCT	CTCGCTGCAG	GAGCTGACGT	770
GGAAGATGAG	CGTGCGGGAC	TGCGCTTGGC	TGCGCAGGAG	CCCAGGTGAG	GAGGTGGTGG	CCGTGAGGG	840
CCCAGGCCCC	AGAGCTGAAT	GCAGTAGGGG	CTCAGAAAAG	GGGGCAGGCA	GAGCCCTGGT	CCTCCTGTCT	910
CCATCGTCAC	GTGGGCACAC	GTGGCTTTTC	GCTCAGGACG	TGAGTGGAC	ACGGTGAICT	CTGCCTCTGC	980
TCTCCCTCCT	GTCCAGTTTG	CATAAACTTA	CG				1012

Fig. 11

GAATTCGCGG CCGCGTCGAC GTTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC CCTGGCCCCG 70
 GCCACCCCCG CGATGCCCGG CGCTCCCCGC TGCCGAGCCG TCGCTCCCT GCTGCGCAGC CACTACCGCG 140
 AGGTGCTGCC GCTGGCCACG TTCGTGCGGC GCCTGGGGCC CCAGGGCTGG CGGCTGGTGC AGCGCGGGGA 210
 CCCGGCGGCT TTCCGCGCGC TGGTGGCCCA GTGCTGGTGC TCGTGGCCCT GGGACGCACG GCCGCCCCCC 280
 GCCGCCCCCT CTTCCGCCA GGTGTCTGCT CTGAAGGAGC TGGTGGCCCG AGTGCTGCAG AGGCTGTGCG 350
 AGCGCGGCGC GAAGAACGTG CTGGCCTTCG GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCGCCCCCGA 420
 GGCCTTCACC ACCAGCGTGC GCAGCTACCT GCCCAACACG GTGACCGACG CACTGCGGGG GAGCGGGGCG 490
 TGGGGGCTGC TGCTGCGCCG CGTGGGCGAC GACGTGCTGG TTCACCTGCT GGCACGCTGC GCGCTCTTTG 560
 TGCTGGTGGC TCCAGCTGC GCCTACCAGG TGTGCGGGCC GCCGCTGTAC CAGCTCGGCG CTGCCACTCA 630
 GGCCCGGCCC CCGCCACACG CTAGTGGACC CCGAAGGCGT CTGGGATGCG AACGGGCGCTG GAACCATAGC 700
 GTCAGGGAGG CCGGGTCCC CCTGGGCCTG CCAGCCCCGG GTGCGAGGAG GCGCGGGGGC AGTGCCAGCC 770
 GAAGTCTGCC GTTGCCCAAG AGGCCCAGGC GTGGCGCTGC CCCTGAGCCG GAGCGGACGC CCGTTGGGCA 840
 GGGGTCTCTG GCCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT GTCACCTGCC 910
 AGACCCGCGG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG CCACTCCCAC CCATCCGTGG 980
 GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC ACCACGTCCC TGGGACACGC CTTGTCCCC 1050
 GGTGTACGCC GAGACCAAGC ACTTCCTCTA CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA 1120
 CTCAGCTCTC TGAGGCCCAG CTGACTGGC GCTCGGAGG TCGTGGAGAC CATCTTCTG GGTTCAGGC 1190
 CCTGGATGCC AGGGAATCCC CGCAGGTGCG CCCGCTGCG CCAGCGCTAC TGGCAAATGC GGCCTCTGTT 1260
 TCTGGAGCTG CTTGGGAACC ACGCGCAGT CCCCTACGG GTGCTCCTCA AGACGCATG CCGCGTCCGA 1330
 GCTGCGGTCA CCCAGCAGC CGGTGTCTGT GCGCGGGAGA AGCCCCAGGG CTCTGTGGCG GCGCCCGAGG 1400
 AGGAGGACAC AGACCCCGT GCGCTGGTGC AGCTGCTCCG CCAGCACAGC AGCCCCCTGGC AGGTGTACGG 1470
 CTTCTGTCGG CCTGCTCTGC GCGCGCTGGT GCGCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC 1540
 TTCCTCAGGA ACACCAAGAA GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT 1610
 GGAAGATGAG CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 1680
 CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT CTCCTGTCT 1750
 CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC ACGGTGATCT CTGCCTCTGC 1820
 TCTCCCTCCT GTCCAGTTTG CATAAACTTA CGAGGTTCAC CTTACGTTT TGATGGACAC GCGGTTTCCA 1890
 GCGCGCCAGG CCAGAGCAGT GAACAGAGGA GGCTGGGCGC GGCAGTGGAG CCGGGTTGCC GGCAATGGGG 1960
 AGAAGTGTCT GGAAGCACAG ACGCTCTGGC GAGGGTGCCT GCAGGGGTTG GCTGTGTTCC GGCCGCAGAG 2030
 CACCGTCTGC GTGAGGAGAT CCTGGCCAAG TTCCTGCACT GGCTGATGAG TGTGTACGTC GTCGAGCTGC 2100
 TCAGGTCTTT CTTTTATGTC ACGGAGACCA CGTTTCAAAA GAACAGGCTC TTTTCTACC GGAAGAGTGT 2170
 CTGGAGCAAG TTGCAAGCA TTGGAATCAG ACAGCACTTG AAGAGGGTGC AGCTGCGGGA GCTGTGCGAA 2240
 GCAGAGGTCA GGCAGCATCG GGAAGCCAGG CCCGCCCTGC TGACGTCCAG ACTCCGCTTC ATCCCCAAGC 2310
 CTGACGGGCT GCGGCCGATT GTGAACATGG ACTACGTCGT GGGAGCCAGA ACGTTCCGCA GAGAAAAGAG 2380
 GGTGGCTGTG CTTTGGTTTA ACTTCCTTTT TAAACAGAAG TGCCTTTGAG CCCACATTT GGTATCAGCT 2450
 TAGATGAAGG GCGCGGAGGA GGGGCCACGG GACACAGCCA GGGCCATGGC ACGGCGCCAA CCCATTTGTG 2520
 CGCACGGTGA GGTGGCCGAG GTGCCGGTGC CTCAGAAAAA GCAGCGTGGG GGTGTAGGGG GAGCTCCTGG 2590
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 TTCAGAGTCT CAAAAGCTGG GACCCTCAGG GCCAAATGGG GCCACACCTT GTCCTGGAAG AAATCATGGT 2800
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 TAATCCTTCC CGGCGAAAAT AGGCTCCACT GGTGTCTGCA ACAAGCGGAG TCTCTAGGCC TGGTCCCTGG 2940
 GGGCAGTGCC ACACATACAC ATACCTTTTC CTCGGCTCCA CAGGTAGCTT GGTGCCCTGC AGGGTGCCAG 3010
 GCGGCCCTC TCCAACACCA GCCAGTGCTG CGATTGCGC AGACCAGGCT CCGGCTGCGT TGATCACAA 3080
 GCGCATTTCC ACAGGCTGGT ACTCCAGGCT GCGGTCCATC TTCACATGGA CTTCAATGAT CCTTTTCAAG 3150
 ACCACCGCTT TGTCTCTGT GGTCAACATG CGTTGAGATG AAGAGACAAA ACGTGTCAAC TCTCCCTGGC 3220
 AGAAAAGGAC TCCCAAGGAC TGGACCTTTC GCCGAAGCCC CTGGAGCAGA CACCAGGGGT CAAACCAACC 3290
 TTCGTCTCTC ATCCCATAG ACGCCAAAGC CACTCCCTCT GTGTTTATCC AGGGAACTT GTTCCGAAGC 3360
 TGATCAGGAG ACATCAGAGA AACTTTGGCT CCTCCTGCC TCTGCACTTT CACGTTGCTC TCCATGGCTG 3430
 CAGCATCCTT TTCTGAAGCC AGCAAGAGGT AGCCCGAGGG GTTGAACCGG AGGTCCAGGG GAGGAGCATC 3500
 GACTACGGCC AGGTACTCAT TGATGTTCCG TAGAAAGCTG GCTGAAAAGA GGGAGAGCTG GATGTTCTCA 3570
 GGCAATGAGA ACTGCTGACA AATCCACCT ACTGAGAGCC CAGTGGAGGC CTGTGAATAC GTGTGGTCCC 3640
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 GCCAAGCACC CCACCTCCCA CGATCACCAC ATCCGAGTGC TCGGGAGGCA GGTGGCTGGT GTCTTGCACT 3780
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 CCAGAGAAAA GCCTCCTCTG CGCGTGCCTG CCCTCCGGGT CAAGAGGCC CGGCCCATGC CGTGCGGCAG 3920
 AACCTCCGA ATCATAGCCC CTCTGAGCCC GGGTCGACGC GGCCGCAAT TC 3972

Fig.12

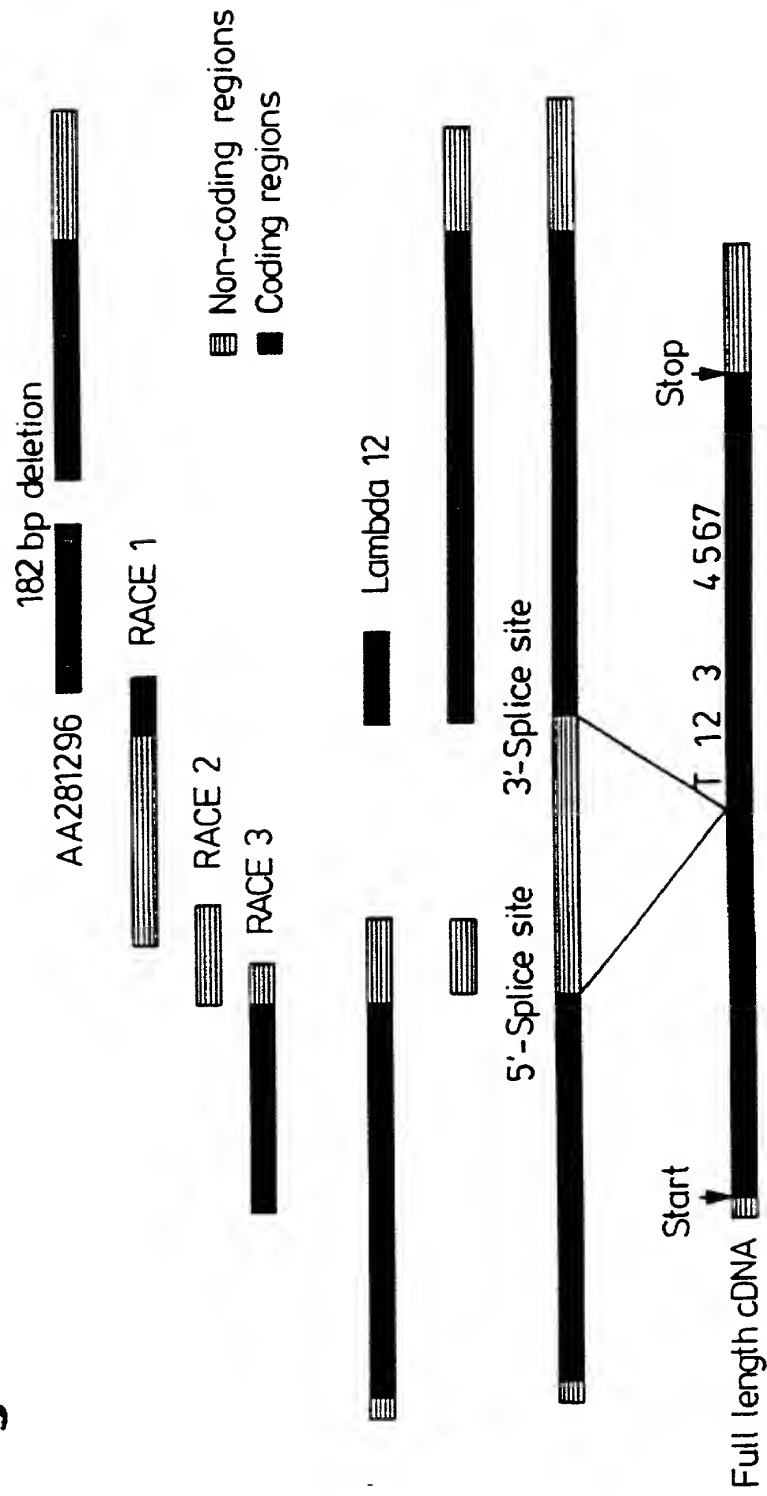
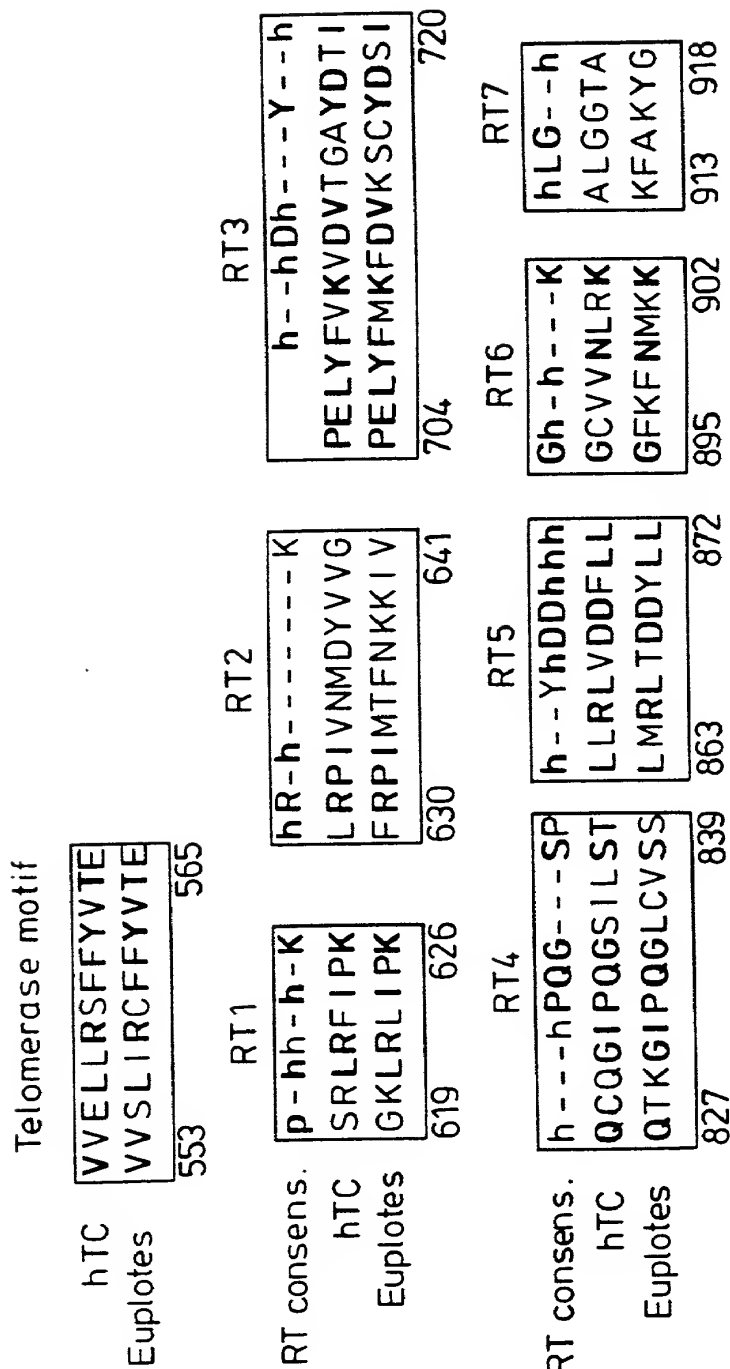


Fig.13



[illegible]

CCGGAAGAGT	GTCTGGAGCA	AGTTGCAAAAG	CATTGGAATC	AGACAGCACT	TGAAGAGGGT	GCAGCTGCCG	1853
GAGCTGTCCG	AAGCAGAGGT	CAGGCAGCAT	CGGGAAGCCA	GGCCCCCCT	GCTGACGTCC	AGACTCCGCT	1923
TCATCCCCAA	GCCTGACGGG	CTGCGGCCGA	TTGTGAACAT	GGACTACGTC	GTGGGAGCCA	GAACGTTCCG	1993
CAGAGAAAAG	AGGGCCGAGC	GTCTCACCTC	GAGGGTGAAG	GCACTGTTCA	GCGTGCTCAA	CTACGAGCGG	2063
GCGCGGCGCC	CCGGCCTCCT	GGGCGCCTCT	GTGCTGGGCC	TGGACGATAT	CCACAGGGCC	TGGCGCACCT	2133
TCGTGCTGCG	TGTGCGGGCC	CAGGACCCGC	CGCCTGAGCT	GTACTTTGTC	AAGGTGGATG	TGACGGGCGC	2203
GTACGACACC	ATCCCCCAGG	ACAGGCTCAC	GGAGGTCATC	GCCAGCATCA	TCAAACCCCA	GAACACGTAC	2273
TGCGTGCGTC	GGTATGCCGT	GGTCCAGAAG	GCCGCCCATG	GGCACGTCCG	CAAGGCCTTC	AAGAGCCACG	2343
TCTCTACCTT	GACAGACCTC	CAGCCGTACA	TGCGACAGTT	CGTGGCTCAC	CTGCAGGAGA	CCAGCCCCTC	2413
GAGGGGTGCC	GTGCTCATCG	AGCAGAGCTC	CTCCCTGAAT	GAGGCCAGCA	GTGGCCTCTT	CGACGTCCTC	2483
CTACGCTTCA	TGTGCCACCA	CGCCGTGCGC	ATCAGGGGCA	AGTCCTACGT	CCAGTGCCAG	GGGATCCCGC	2553
AGGGCTCCAT	CCTCTCCACG	CTGCTCTGCA	GCCTGTGCTA	CGGCGACATG	GAGAACAAAGC	TGTTTGCGGG	2623
GATTCGGCGG	GACGGGCTGC	TCCTGCGTTT	GGTGGATGAT	TTCTTGTTGG	TGACACCTCA	CCTCACCAC	2693
GCGAAAACCT	TCCTCAGGAC	CCTGGTCCGA	GGTGTCCCTG	AGTATGGCTG	CGTGGTGAAC	TTGCGGAAGA	2763
CAGTGGTGAA	CTTCCCTGTA	GAAGACGAGG	CCCTGGGTGG	CACGGCTTTT	GTTCAGATGC	CGGCCACCG	2833
CCTATTCCCC	TGGTGCGGCC	TGCTGCTGGA	TACCCGGACC	CTGGAGGTGC	AGAGCGACTA	CTCCAGCTAT	2903
GCCCGGACCT	CCATCAGAGC	CAGTCTCACC	TTCAACCGCG	GCTTCAAGGC	TGGGAGGAAC	ATGCGTCGCA	2973
AACTCTTTGG	GGTCTTGCGG	CTGAAGTGTC	ACAGCCTGTT	TCTGGATTTG	CAGGTGAACA	GCCTCCAGAC	3043
GGTGTGCACC	AACATCTACA	AGATCCTCCT	GCTGCAGGCG	TACAGGTTTC	ACGCATGCGT	GCTGCAGCTC	3113
CCATTTTCATC	AGCAAGTTTG	GAAGAACCCC	ACATTTTTCC	TGCGCGTCAT	CTCTGACACG	GCCTCCCTCT	3183
GCTACTCCAT	CCTGAAAGCC	AAGAACGCAG	GTATGTGCAG	GTGCCTGGCC	TCAGTGGCAG	CAGTGCCTGC	3253
CTGCTGGTGT	TAGTGTGTCA	GGAGACTGAG	TGAATCTGGG	CTTAGGAAAGT	TCTTACCCCT	TTTCGCATCA	3323
GGAAAGTGGT	TAACCCAACC	ACTGTCAGGC	TCGTCTGCCC	GCCCTCTCGT	GGGGTGAGCA	GAGCACCTGA	3393
TGGAAGGGAC	AGGAGCTGTC	TGGGAGCTGC	CATCCTTCCC	ACCTTGCTCT	GCCTGGGGAA	GCCTGGGGGG	3463
GCCTGGTCTC	TCCTGTTTGC	CCATGGTGG	GATTTGGGGG	GCCTGGCCTC	TCCTGTTTGC	CCTGTGGTGG	3533
GATTGGGCTG	TCTCCCGTCC	ATGGCACTTA	GGGCCCTTGT	GCAAACCCAG	GCCAAGGGCT	TAGGAGGAGG	3603
CCAGGCCCCAG	GCTACCCAC	CCCTCTCAGG	AGCAGAGGCC	GCGTATCACC	ACGACAGAGC	CCCGCGCCGT	3673
CCTCTGCTTC	CCAGTCACCG	TCCTCTGCCC	CTGGACACTT	TGTCCAGCAT	CAGGGAGGTT	TCTGATCCGT	3743
CTGAAATTCA	AGCCATGTG	AACCTGCGGT	CCTGAGCTTA	ACAGCTTCTA	CTTTCTGTTT	TTTCTGTGTT	3813
GTGGAGACCC	TGAGAAGGAC	CCTGGGAGCT	CTGGGAATTT	GGAGTGACCA	AAGGTGTGC		

Fig. 15

Fig. A

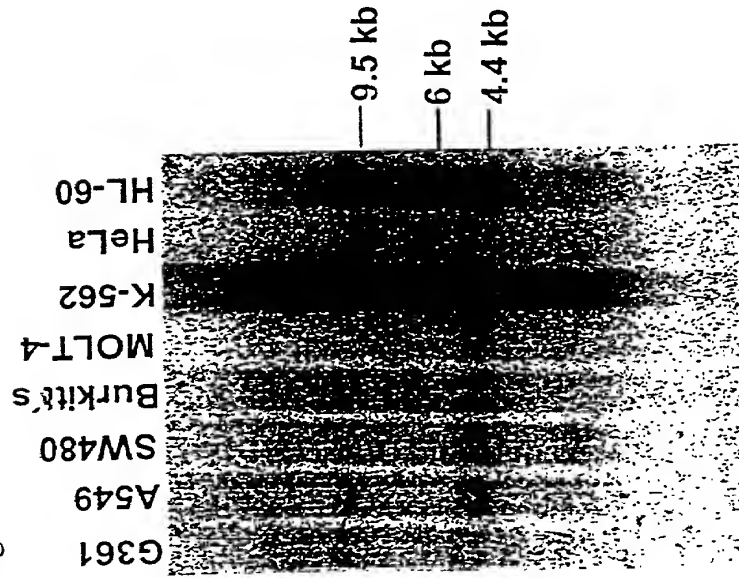


Fig. B

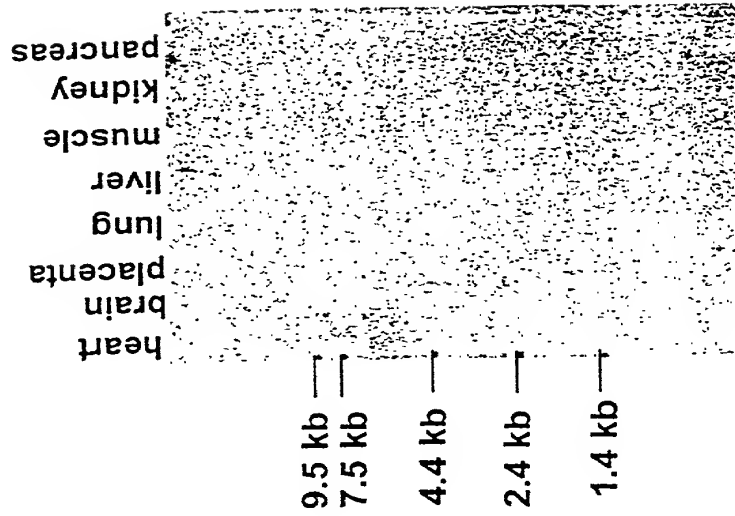
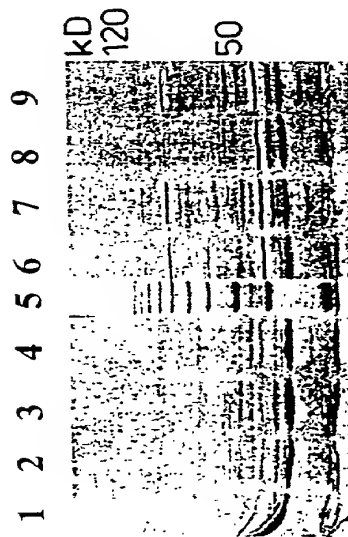
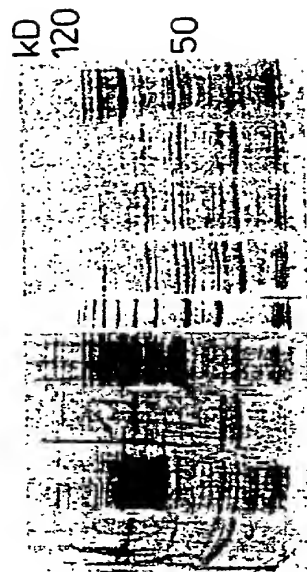


Fig. A

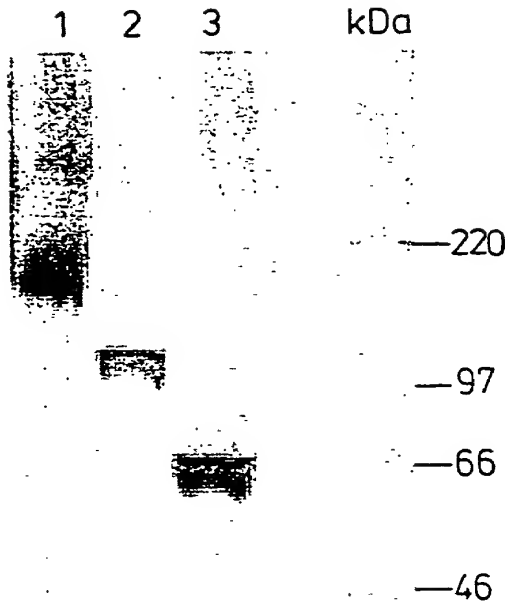
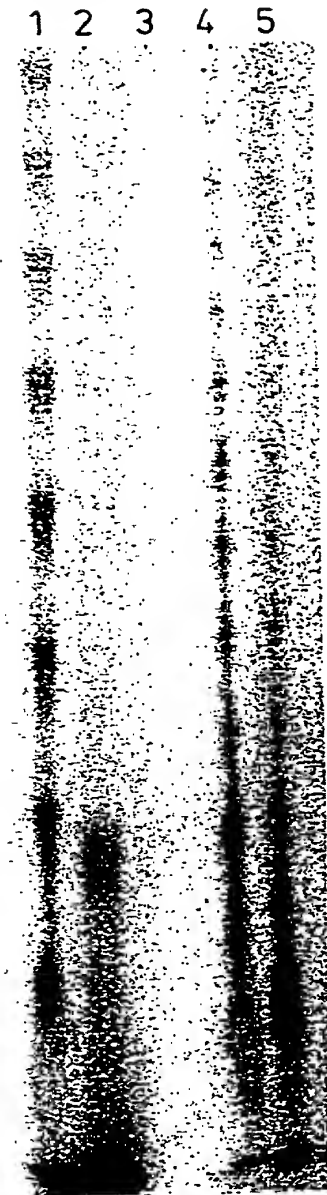


ipMAL EST	ipMAL AI	pMAL AI	M EST	ipMAL EST	pMAL AI	pMAL AI
-	+	-	+	-	+	+
anti-B pre-immun			anti-C pre-immun			

1 2 3 4 5 6 7 8 9



pMAL- EST	pMAL- EST	pMAL- AI	pMAL- AI	pMAL- EST	pMAL- EST	pMAL- AI	pMAL- AI
-	+	-	+	-	+	-	+
anti-B ¹⁰ immun.				anti-C ¹⁰ immun.			

Fig. 17**Fig. 19****Fig. 18**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS DIAGNOSTIC AND THERAPEUTIC USE

the specification of which is attached hereto,

or was filed on **June 9, 1998**

as a PCT Application Serial No. **PCT/EP98/03468**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s), the priority(ies) of which is/are to be claimed:

197 26 329.1 (Number)	Germany (Country)	June 20, 1997 (Month/Day/Year Filed)
198 13 274.3 (Number)	Germany (Country)	March 26, 1998 (Month/Day/Year Filed)
198 16 496.3 (Number)	Germany (Country)	April 14, 1998 (Month/Day/Year Filed)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose the material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

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POST OFFICE ADDRESS c/o Bayer Aktiengesellschaft, D 51368 Leverkusen, Germany			
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POST OFFICE ADDRESS c/o Bayer Aktiengesellschaft, D 51368 Leverkusen, Germany			
FULL NAME OF SIXTH INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SEVENTH INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF EIGHTH INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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